UNIVERSITÀ DEGLI STUDI DI MILANO – BICOCCA

Department of Earth and Environmental Sciences (DISAT)

International Master's Degree in Marine Sciences

The Effects of Extreme Low-Light Stress on the Coral *Pachyseris speciosa* from Singapore's Turbid Reef: a Photo-Physiological Approach

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Student Number 842419

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ABSTRACT

Tropical coral reefs are among the most productive ecosystems on our planet. They host an extraordinary biodiversity, and they play a fundamental role both at an economic and social level, providing food and protection to many populations living on the coast. Unfortunately, in the last decades, they have been threatened by a plethora of environmental and anthropogenic stressors, such as increased sedimentation, sea-level rise, and ocean acidification. In Singapore, land reclamation activities, terrestrial runoff, and routine dragging of shipping routes, have been conducted since the 19th century, contributing to an increase in sedimentation rates. Consequently, the ability of photosynthetic active radiations (PAR) to penetrate in the water column decreased, affecting coral survivorship, and shifting the depth of their ecological niches.

This work, through the integration of photo-physiological data, aims to increase our understanding of *Pachyseris speciosa*, an ecologically important reef-building species, to assess if it could be used as a target species for coral restoration projects deeper than 6m and if it could resist to further increase sedimentation from increasing reclamation events. Therefore, *Pachyseris speciosa* nubbins were exposed to three different light treatments for four weeks: Control (C); ~35 µmol m^{-2} s^{-1}, mimicking their normal niche conditions, Intermittent Light (IL); two days of normal light and five days of complete darkness, and Dark (D); complete darkness. Throughout the experiment the following parameters were monitored: zooxanthellae density, chlorophyll a concentration, colour score, maximum quantum yield (MQY), effective quantum yield (EQY), and rapid light curves (RLCs).

Results of this study bring evidence that *Pachyseris speciosa* is extremely resistant to extreme low-light conditions and even complete darkness. In fact, at the end of the experiment, though a decrease in zooxanthellae density, chlorophyll a concentration, and colour was measured, no mortality was recorded. Moreover, *Pachyseris speciosa* showed acclimation responses in Intermittent Light treatment through increased effective quantum yield (EQY), decreased electron transport rate max (ETR_{max}), and decreased minimum saturation irradiance (E_k). This suggests that this species can cope with increasing sedimentation and low light intensity.

Overall, this study showed plasticity in the photosynthetic responses of *Pachyseris speciosa* to acute low-light conditions and established baseline data on its capacity to tolerate low-light conditions. Finally, this coral is suggested as a good candidate for coral restoration projects in this region.

RIASSUNTO

Le scogliere coralline tropicali sono tra gli ecosistemi più produttivi del nostro pianeta. Ospitano una straordinaria biodiversità e svolgono un ruolo fondamentale sia a livello economico che sociale, fornendo cibo e protezione a molte popolazioni che vivono sulla costa. Sfortunatamente, negli ultimi decenni, sono stati minacciati da una pletora di fattori di stress ambientali e antropogenici, come l'aumento della sedimentazione, l'innalzamento del livello del mare e l'acidificazione degli oceani. A Singapore, le attività di bonifica del terreno, il deflusso terrestre e il dragaggio ricorrente delle rotte marittime, sono stati condotti dal 19° secolo, contribuendo ad un aumento dei tassi di sedimentazione. Di conseguenza, la capacità delle radiazioni attive fotosintetiche (PAR) di penetrare nella colonna d'acqua è diminuita, influenzando la sopravvivenza dei coralli e modificando la profondità delle loro nicchie ecologiche.

Questo lavoro, attraverso l'integrazione di dati foto-fisiologici, mira ad aumentare la nostra comprensione su *Pachyseris speciosa,* una specie ecologicamente importante che costruisce la scogliera corallina, per valutare se possa essere utilizzata come specie target per progetti di ripristino dei coralli a profondità maggiori di 6 metri e se possa resistere all'aumento della sedimentazione dovuto a crescenti eventi di bonifica. Pertanto, i frammenti di *Pachyseris speciosa* sono stati esposti a tre diversi trattamenti luminosi per quattro settimane: Controllo (C), ~35 µmol m^{-2} s^{-1}, imitando le loro normali condizioni di nicchia ecologica; Luce intermittente (IL), due giorni di luce normale e cinque giorni di completa oscurità; Buio (D), completa oscurità. Durante l'esperimento sono stati monitorati i seguenti parametri: densità delle zooxantelle, concentrazione di clorofilla a, punteggio di colore, resa quantica massima (MQY), resa quantica effettiva (EQY) e curve di luce rapida (RLCs).

I risultati di questo studio dimostrano che *Pachyseris speciosa* è estremamente resistente a condizioni di scarsa illuminazione e persino alla completa oscurità. Infatti, alla fine dell'esperimento, sebbene sia stata rilevata una diminuzione della densità delle zooxantelle, della concentrazione di clorofilla a e di colore, non è stata registrata alcuna mortalità. Inoltre, *Pachyseris speciosa* ha mostrato risposte di acclimatazione nel trattamento con Luce Intermittente attraverso un aumento della resa quantica effettiva (EQY), una diminuzione della velocità massima di trasporto degli elettroni (ETR_{max}) e una diminuzione dell'irradianza minima di saturazione (E_k). Ciò suggerisce che questa specie è in grado di far fronte all'aumento della sedimentazione e alla bassa intensità luminosa. Nel complesso, questo studio ha mostrato plasticità nelle risposte fotosintetiche di *Pachyseris speciosa* in condizioni di scarsa illuminazione acuta e ha stabilito dati di riferimento sulla sua capacità di tollerare condizioni di scarsa illuminazione. Infine, questo corallo è suggerito come buon candidato per i progetti di restaurazione di coralli in questa regione.

1. INTRODUCTION

1.1 Corals

The so-called hermatypic corals are animals belonging to the phylum Cnidaria that represent the main coral reef-building organisms. The majority of them are members of the class Anthozoa, subclass Hexacorallia, and order Scleractinia and for this reason are called scleractinians. They are colonial organisms, and the animal called polyp acts as their fundamental unit (Titlyanov and Titlyanova, 2020).

1.1.1 Biology and Anatomy

A polyp's anatomy is comparable to the structure of an upside-down jellyfish, with an oral disc and a disc that anchors the body to the basal plate, the skeleton. They are soft-bodied animals characterised by a biradial symmetry. Their tissue is composed by two layers (epidermis and gastrodermis or endodermis) separated by a thin connective-tissue layer (mesoglea) (Fig. 1.1). Moreover, they have only one opening, the mouth, which provides a direct exchange of water, particulate food, and waste between the gastrovascular system and the external seawater and is surrounded by tentacles containing stinging cells called nematocysts (Odum and Odum, 1955). A pharynx connects the central mouth, encircled by tentacles, to the stomach cavity (Titlyanov and Titlyanova, 2020). The skeletal counterpart to a polyp is the chalice, the cavity occupied by the polyp (Rosen, 1986). This hard cup-shaped skeleton is also called corallite. It is secreted by the polyp’s lower epidermal layer, it is made of calcium carbonate and it is composed of several basal plates, and it also has a protective function (Odum and Odum, 1955). The gastrodermis is used for the digestion of zooplankton caught by the tentacles that host specialized stinging cells called nematocysts, which discharge an arrow-like barb and a toxin that stuns their microscopic preys (Goreau et al., 1979; Veron, 1986; Levinton, 2008). Interestingly, genetically identical polyps (clones) are linked by a common gastrovascular system and constitute the whole coral colony, even though some solitary single-polyp forms exist (e.g. *Fungia* spp.) (Odum and Odum, 1955).

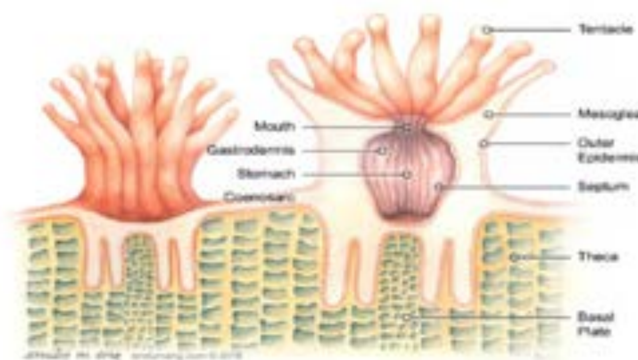


Fig. 1.1: Anatomy of a coral polyp (Emily M. Eng)

1.1.2 Coral – Zooxanthellae Symbiosis and Calcification

Scleractinian corals coexist in symbiosis with zooxanthellae, which are hosted in the polyp tissues and in particular in the endodermis. These small unicellular algae (8-12 µm) are dinoflagellates of the family *Symbiodiniaceae* (LaJeunesse et al., 2018), and they are photosynthetically competent, being able to fix carbon dioxide and release oxygen, providing nutrients for the coral's cells (Venn et al., 2008). Each member of this symbiotic relationship gains from the other's presence, making this relationship mutualistic. The algae supply up to 90% of the energy required for the coral's maintenance while the coral protects and feeds the algae (Kaiser et al., 2005).

Zooxanthellae are autotrophic photosynthetic organisms that fix carbon using light, CO_2, and micronutrients. In addition, other than using light, they can also use waste from other sources to perform photosynthesis or they can get it from the environment around them (Kaiser et al., 2005). The coral provides a protected environment and supplies carbon dioxide (CO_2) and nutrients (nitrogen and phosphorus) from cellular respiration, which are necessary for photosynthesis (Yellowlees et al., 2008). In exchange, the coral polyp host receives oxygen (O_2) and carbon in the form of sugars, amino acids, and lipids from the zooxanthellae through photosynthesis (Fig. 1.2). These nutrients help corals maintain their metabolism, develop, calcify, reproduce, and survive (Davy et al., 2012; Yellowlees et al., 2008). This symbiotic relationship explains the tight nutrient recycling which can generate the high productivity typical of this ecosystem (Hoegh-Guldberg, 1999).

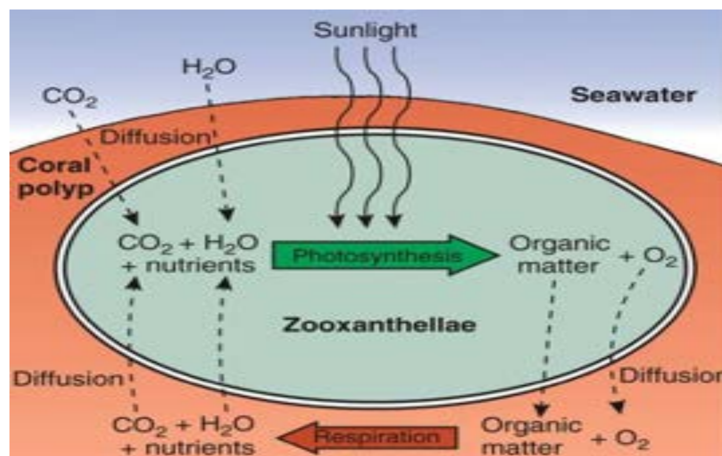
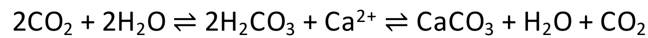


Fig. 1.2: Movement of molecules between coral tissue and zooxanthellae (Castro and Huber, 2015)

This symbiosis is also a crucial component of the creation and deposition of the coral's $CaCO_3$ skeleton and, consequently, the development of coral reefs. Indeed, corals use calcium and bicarbonate ions to generate $CaCO_3$ skeletons from the CO_2 that is produced as a byproduct of photosynthesis by zooxanthellae. The constant removal of CO_2 by zooxanthellae tips the chemical

balance in favour of producing more $CaCO_3$, allowing corals to quickly secrete skeleton and form reefs. Hence, corals require zooxanthellae to develop quickly and large enough to construct reefs and keep up with bioerosion for skeleton production to outpace erosion (Veron, 2011). Algal photosynthesis removes CO_2 from the calcifying environment, causing a net mass-action effect that shifts the carbonate equilibrium and speeds up the precipitation of $CaCO_3$, according to the inorganic reactions that follow. This is the most well-known contribution of endocytic algae to coral calcification (Goreau, 1959):



$$2CO_2 + 2H_2O \rightleftharpoons 2H_2CO_3 + Ca^{2+} \rightleftharpoons CaCO_3 + H_2O + CO_2$$

The process of calcification modifies the seawater Dissolved Inorganic Carbon (DIC) system by releasing 0.6 moles of CO_2 (Frankignoulle et al., 1994) and removing 2 moles of bicarbonate (Smith and Key, 1975) for each mole of calcium carbonate precipitated. $CaCO_3$ dissolution has the opposite effect on the DIC system. Theoretical considerations and experiments performed on calcifying organisms indicate that calcification is regulated by the seawater DIC chemistry. Indeed, the availability of CO_3^{2-} plays a critical role in controlling the calcium carbonate precipitation (Kleypas et al., 1999). Therefore, calcification is also modified by changes in DIC induced by the steady increase of atmospheric pCO_2, due to anthropogenic inputs (Houghton et al., 1996). Hereinafter, the dissociation of carbon dioxide in water:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2H^+$$

1.1.3 General Classification of Corals

Corals can be mainly classified based on their calcification process into two groups: "soft corals" and "stony (or hard) corals". Soft corals do not produce a rigid $CaCO_3$ skeleton, although they do secrete small calcareous granules called sclerites (Alderslade and Fabricius, 2008). They mainly belong to the subclass Octocorallia. On the contrary, stony corals (subclass Hexacorallia, order Scleractinia) are responsible for reef formation through the production and secretion of calcium carbonate skeleton (e.g., $CaCO_3$, or limestone) (Alderslade and Fabricius, 2008).

Corals can also be classified into different types based on their morphology: common descriptive terms include branching, columnar, massive, foliose, laminar, encrusting, and free-living (Veron, 2000). This classification accurately predicts three aspects of coral reef conservation value: coral species richness, rare species occurrence, and habitat complexity, as measured by coral morphological diversity (Roberts and Ormond, 1987).

1.1.4 Reproduction

Corals can either be gonochoric, where the colony generate either sperm or eggs, or hermaphroditic, where the same colony produces both egg and sperm cells (also known as

gametes). The development of the egg and sperm in hermaphroditic corals can be synchronous or sequential (develop egg and sperm at different times) (Harrison, 2011).
There are two modes of sexual reproduction: broadcast spawning (external fertilisation) and brooding (internal fertilisation). Most coral species use broadcast spawning, releasing eggs and sperm into the water, which leads to the development of planula larvae and colony formation. On the other hand, brooding corals fertilise internally, and release planulae already associated with symbiotic algae only when they are ready for settlement (Baird et al., 2009), so their reproduction is asexual. In general, fertilized eggs develop into planula larvae, settle, and form colonies (Martinez and Abelson, 2013). Successful coral recruitment is supported when competition from algae is limited and there is low predation, high light availability, and low sedimentation (Porter and Tougas, 2001). The principal modes of asexual reproduction in corals are: fragmentation, budding (intratentacular and extratentacular), fission, and bailout (Carpenter, 1979; Khaled bin Sultan, 2023):

- **Fragmentation**: when a piece of coral intentionally or unintentionally (storms, human disturbance, etc.) is broken off from the parent coral. It can grow, developing into a mature coral and starting new colonies. This method is often used in coral reef restoration
- **Budding**: when a portion of the parent polyp pinches off to form a new individual. There are two ways in which this occurs:
    - Intra-tentacular: buds form from the parent polyp's oral disks, producing same-sized polyps within the ring of tentacles (Fig. 3a)
    - Extra-tentacular: buds form outside the parent polyp's ring of tentacles, producing a smaller polyp (Fig. 3b)

Fig. 1.3: a) Intra-tentacular budding, b) Extra-tentacular budding (Khaled bin Sultan, 2023)

- **Fission**: when solitary corals decalcify or break up, their skeletons, creating two pieces and then growing their other half back (Khaled bin Sultan, 2023)

- **Bailout**: when a single polyp abandons its colony and settles on the substrate to create a new colony. Sometimes this is due to a stressful event such as coral bleaching (Sammarco, 1982)

The host can acquire its symbionts either from its parents (vertically) or from the surrounding environment (horizontal) (Barneah et al., 2007; Huang et al., 2008). Vertical transmission assures maximum fit between the host and the symbiont, however, can be disadvantageous. On the other hand, horizontal transmission maintains higher diversity and ensures the survival of the symbiosis under different environmental conditions (Stat et al., 2008).

1.2 The Coral Reefs Ecosystems

1.2.1 Distribution and Diversity

Coral reefs are massive structures made of limestone deposited by coral polyps (NOAA, 2023). Their cover was estimated to be around 284,300 km^2, with 91% of this area in the Indo-Pacific region (Fig. 1.4.). Together with other animals like coralline algae, corals' active growth, over decades or millennia, has resulted in the formation of these enormous carbonate structures (Spalding, et al., 2001). Temperature, salinity, sedimentation, and light play major roles in determining where the reef-building corals grow. Shallow-water reef corals are indeed found mainly in the top 100 m of the water column between the latitudes 25°N and 25°S, in a temperature range from 18 to 30°C and salinity between 32% and 40% (Veron, 1986). In addition, the optimal calcification rate occurs at 27 °C (Fabricius et al., 2011). From a physical standpoint, coral reefs are crucial for preventing storm damage, erosion, and flooding along the shoreline by minimizing wave action, permitting the growth of associated ecosystems like mangroves and seagrass beds, and offering habitat to a variety of species (Hoegh-Guldberg, 1999), they can also function as spawning, breeding, and feeding sites, creating ecological niches (Moberg and Folke, 1999). Interestingly, reefs are a far more effective wave barrier than any human-made sea wall. Moreover, this allows a deposition of sand alongside the coast rather than an erosion (Goreau et al., 2013). Over the past several decades, biogeographers have proposed centres of marine biodiversity of varying shapes, all centred on the Indonesian-Philippines Archipelago (Fig. 1.4), and they have named it: the Western Pacific Diversity Triangle (Hoeksema, 2007).

Fig. 1.4: Distribution and diversity of reef-building scleractinian corals (Veron, 2000).

The Coral Triangle defined by *Coral Geographic* is an area of 5.5 10^6 km^2 of ocean territory of Indonesia, the Philippines, Malaysia, Timor Leste, Papua New Guinea, and the Solomon Islands. It represents less than 1.6% of the world's total ocean area (Veron, 1995) and it is defined as an area that contains a high proportion of the species diversity of the Indo-Pacific small enough to permit meaningful conservation (Dubinsky and Stambler, 2011). In total, the Coral Triangle has 605 zooxanthellate coral species of which 66% are common to all ecoregions. This diversity amounts to 76% of the world's total species complement. The region also hosts 52% of Indo-Pacific reef fishes (37% of reef fishes of the world) (Allen, 2008; Veron et al., 2009).

1.2.2 Ecological and Economic Role

Coral reefs are significant to the world and human society due to their size and their contributions to biodiversity and productivity on a global scale (Sheppard et al., 2017). Even though they occupy less than 0.1% of the world's ocean floor, coral reefs host more than 25% of all known marine fish species, approximately 70,000 (Bryant et al., 1998; Spalding et al., 2001; Fisher et al., 2015). Reaka-Kudla, 1997 estimated that coral reef biodiversity amounts to about 93,000 described species. However, she then speculated that, if similar ecological and evolutionary processes operate on coral reefs as in rain forests, the number of coral reef species would be "about 600,000 - 950,000 species". In addition, they are among the most diverse and complex of all ecosystems; thus, the most heavily utilized and economically valuable to humankind (Spalding et al., 2001; Burke et al., 2011).

The majority of the world's poorest people are located around the coast and directly rely on reef species for their protein requirements (Pomerance, 1999) as well as for monetary income through commercial fishing and tourism (Moberg & Folke, 1999). Coral reefs are indeed a major attraction for snorkelers, scuba divers, and tourists in general (Bryant et al., 1998). The ecological and socioeconomic importance of coastal marine systems is among the highest on the globe. An estimated US$14 trillion worth of ecosystem products (food and raw materials) and services (disturbance regulation and nutrient cycling) are provided annually by marine ecosystems,

accounting for 43% of the global total (Costanza et al., 1997). Moreover, marine natural compounds derived from reef species have been already identified and shown to have medicinal potential (Carte, 1996; Cerri et al., 2022). Moreover, corals have many functions in coastal erosion management dissipating wave energy. Their shape and structure act as a barrier that provides a natural submerged breakwater (Hettiarachchi et al., 2013). Yet, anthropogenic global climate change poses a hazard to coastal marine ecosystems. So, despite their enormous size, reefs are among the maritime habitats that are most sensitive to human disturbance, making them one of the most endangered marine environments (IPCC, 2001).

1.2.3 Threats

Natural threats to coral reefs can be categorised as short-term and long-term concerns. On a geological timescale, the primary threats are thermal expansion of seawater and glacier melting are the main contributors to sea level rise. Natural hazards on a short time scale could also include cyclonic storms, hurricanes, freshwater flooding, exposure during periodic low tides, predation outbreaks, and disease outbreaks (Wilkinson, 1999). The consequences of ocean-wide changes in water temperature and acidity are also having an impact on corals, and there is strong evidence that reefs already under stress from local variables are more vulnerable to global change (Pandolfi et al., 2005). Corallivorous organisms may also pose a significant stressor due to the pressure of predation or because they may act as disease carriers. The cushion star *Culcita spp.*, the sea snail *Drupella spp.*, and the crown-of-thorn starfish *Acanthaster planci* are among the most notable. The rate of deterioration and erosion of coral reefs around the world can be accelerated by these voracious predators, which are significant biotic stressors. They can also have an impact on the resilience and capacity of the reefs to rebound after stress (Birkeland and Lucas, 1990; Morton et al., 2002; Saponari et al., 2018; Montalbetti et al., 2019). *Drupella spp.* and other corallivorous gastropods may speed up the spread of coral disease (Nicolet et al., 2013; Gignoux-Wolfsohn et al., 2012). Other coral disease vectors are the fireworms (Sussman et al., 2003), and butterflyfish (Aeby, 2007). In dense coral communities, coral predation may aid in the spread of illness. Pathogens can be transmitted by predators through their mouths or feces (Aeby and Santavy, 2006; Rotjan and Lewis, 2008). For example, black band disease is thought to flourish in the presence of corallivorous fishes (Nicolet et al., 2018). Similar to human populations, coral species with higher local abundances may be more susceptible to disease (Willis et al., 2004; Page and Willis, 2008). For example, the coral disease white syndrome has increased in areas where coral cover is high (Willis et al., 2004). Other than natural, threats to coral reefs can also be human-induced; every square meter of water is affected by some anthropogenic driver of ecological change, and a significant portion (41%) is highly affected by multiple (Halpern et al., 2008). There

are many anthropogenic threats, which can be divided in local or global scale. At a local scale, there are, among others, destructive and non-sustainable fishery and tourism practices, over-exploitation, biological invasion, sedimentation, organic and inorganic pollution, mining, and dredging (Cesar, 2000). Severe reef degradation can also be caused from different local threats such as: dynamite fishing, overfishing for both food and aquarium purposes, elevated nutrient runoff, pollution, sedimentation, and direct physical damage from boats and tourists (Gardner et al., 2003; Bruno and Selig, 2007). The combination of so many threats has led to rapid declines in coral populations (Gardner et al., 2003; Bruno and Selig, 2007), also because reefs cannot keep up with their recovery at this fast rate (Hoey et al., 2016). Fish and invertebrate species are likewise impacted by changes in the composition of coral communities and loss of coral cover. After severe coral loss events in the Great Barrier Reef (Sikkel et al., 2019), Tanzania (Garpe et al., 2006), and the Seychelles (Graham et al., 2006), significant declines in fish populations have been observed.

1.2.4 Coral Bleaching

Even though coral reefs have endured throughout geological time, they are currently among the most vulnerable marine ecosystems. Due to the relatively stable tropical habitat in which they have evolved, this ecosystem may be impacted by even small environmental changes that occur too quickly for the opportune genetic adjustments (Levy et al., 2006). Corals can withstand only a limited range of environmental conditions. Under certain conditions, such as increased sea surface temperatures, acidity, sedimentation, or pollution, the relationship between coral polyps and *Symbiodinium* becomes dysfunctional, and the algae become stressed and produce harmful compounds that can damage the coral's tissues. This leads to coral bleaching, which has become a major threat to coral reefs worldwide (Hughes et al., 2018), so, an expulsion of these algae from coral tissue (Fig. 1.5). Bleaching events are becoming more frequent and lasting longer due to an increase in these anomalies brought on by climate change (Dilworth et al., 2020). The symbiont types within a coral colony can greatly affect its ability to adapt to changing environmental conditions (Thornhill et al., 2013).

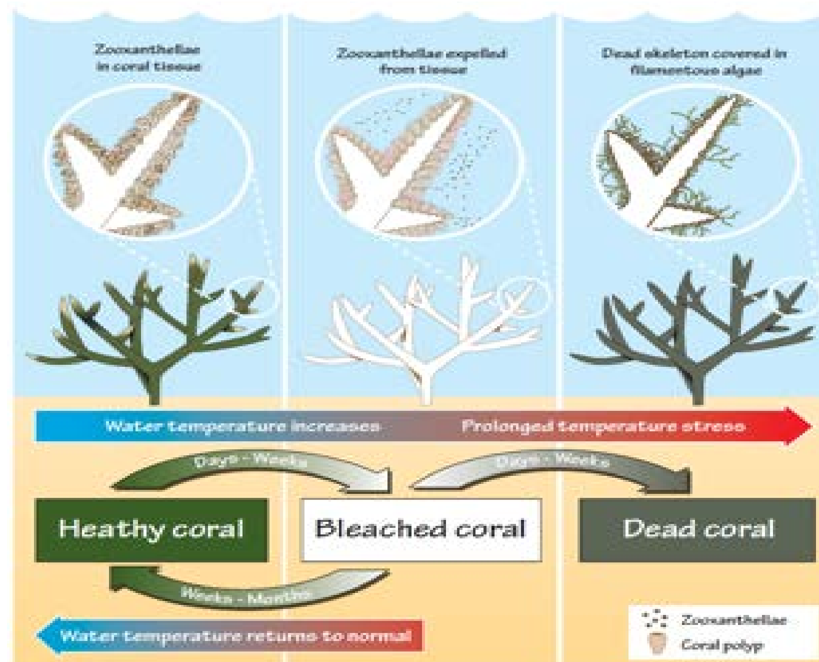


Fig. 1.5: Diagram of thermal coral bleaching (Marshall and Schuttenberg, 2006)

Coral bleaching is the most evident signal of distress in corals. The word "bleaching" refers to the white colour that corals acquire when their symbionts are lost. Microalgae give corals their various colours (Dobson et al., 2021), once lost the symbiosis, the underlying white skeleton can be seen through the transparent remaining tissue (Glynn, 1993; Brown, 1997). Bleached and recovered corals must rely on substitute sources of fixed carbon since they lack their zooxanthellae, which can supply the coral animal with up to 90% of its Daily Metabolic Energy (DME) requirements (Kaiser et al., 2005). Stored energy reserves and heterotrophy are two such alternative sources. While certain coral species (Fitt et al., 2000; Porter et al., 1989) significantly deplete their energy stores during bleaching, other coral species do not (Grottoli et al., 2004).

Thermal stress and UV radiations are among the main causes of coral bleaching; however, other stressful environmental conditions could promote symbiosis disruption, such as nitrogen and phosphate (Pogoreutz et al., 2017; Rosset et al., 2017). Moreover, overwhelming UV radiations, triggers a molecular mechanism which leads to excessive production of pro-oxidant molecules or Reactive Oxygen Species (ROS) (Freeman, 1982; Oakley et al., 2018) and can eventually lead to apoptosis or necrosis (Cziesielski et al., 2019) damaging the photosynthetic machinery of the symbiont (Weis, 2008). Yet, given that different corals exhibit varying degrees of physiological resilience to environmental stress, coral vulnerability to stress and bleaching appears to be highly variable (Obura, 2001; McClanahan et al., 2007; Montano et al., 2010). Finally, mass bleaching events refer to observations of bleached corals spanning hundreds or even thousands of kilometres, thereby affecting entire ecosystems. The frequency and severity of mass bleaching events have been increasing over the last few decades and it is expected to occur more often since

sea-surface temperatures continue to rise due to global climate change (Pandolfi et al., 2011; Hughes et al., 2018) (Fig. 1.6).

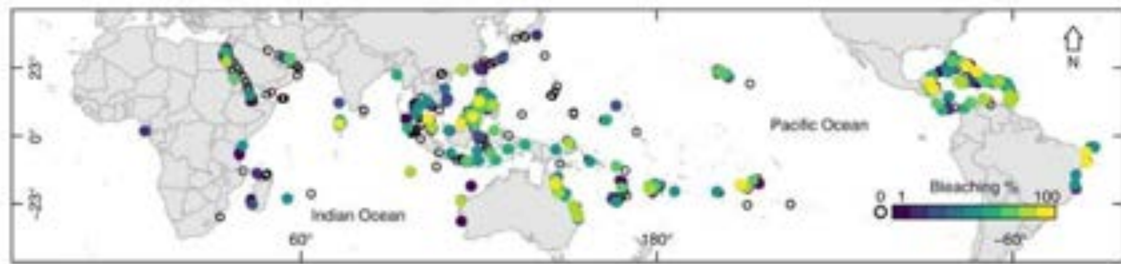


Fig. 1.6: Distribution of coral bleaching globally (Sully et al., 2019)

Interestingly, there have been observed trade-offs in different traits of coral colonies, such as the trade-off between coral colony growth rates and stress tolerance. Branching corals, for example, are fast-growing colonies, but appear to be more susceptible to bleaching than massive and encrusting colony morphologies (Loya et al., 2001). Massive corals are slower growing but may be more resistant to bleaching due to greater tissue thickness and greater ability to efficiently remove cellular toxins (Loya et al., 2001).

1.2.5 Conservation

Nowadays, there is a growing recognition that more holistic and integrated approaches are needed to address the complex and interconnected challenges facing coral reefs, such as the need to balance conservation goals with the needs and interests of local communities and industries (Ban et al., 2017). Many conservation and management approaches aim to address multiple stressors simultaneously, through strategies such as the establishment of marine protected areas, the promotion of sustainable fishing practices, and the reduction of pollution inputs from land-based sources (Cinner et al., 2014). Moreover, different coral restoration techniques have been used in the past years. Two main categories have been considered: asexual propagation such as direct transplantation, nursery-framed coral fragments, and electrochemical methods; sexual propagation such as cultivation and rearing larvae, inoculation of zooxanthellae, or substrata for settlements of coral larvae (Omori, 2019). However, often asexual techniques use fragments with limited genetic diversity which constrains their opportunity of fertilisation and resistance to disease and future stress disturbances (Edwards, 2010; Barton et al., 2017). The main aims of Marine Protected Areas (MPAs) identified in the International Union for Conservation of Nature (IUCN) guidelines for establishing marine protected areas (Kelleher and Kenchington, 1992), are to maintain essential ecological and life-support systems, ensure the sustainable utilisation of species and ecosystems, and preserve biotic diversity (Huete Stauffer et al., 2011). Some of the numerous benefits provided by MPAs are the increased habitat heterogeneity at the seascape level,

biodiversity and raised abundance of species of particular interest for their rarity and cultural value (Terlizzi et al., 2004) as well as opportunities for environmental education and recreation, and can provide aesthetic experiences (Edgar et al., 2007).

1.3 Climate Change

Climate change is a long-term change in the average weather patterns that have come to define Earth's local, regional, and global climates (NASA, 2023). The United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as "a change of climate that is attributed directly or indirectly to human activity, that alters the composition of the global atmosphere, and that is in addition to natural climate variability over comparable periods." By contrast, the Intergovernmental Panel on Climate Change (IPCC) defines climate change broadly as "any change in climate over time whether due to natural variability or as a result of human activity" (Pielke, 2004). In general, this phenomenon is modifying world's ocean properties at an unprecedented rate (Gattuso et al., 2015), triggering the loss of biodiversity and deeply affecting tropical coral reefs. Before 2008, 24% of the world's reefs were threatened by anthropogenic stress, and another 20% had already suffered irreparable damage from global warming (Wilkinson, 2008). More recent projections, predict a coral reef decline across the globe equal to 70-80% (Hoegh-Guldberg, 2018). Temperature increase, sea level rise, which will lead to changes in ocean circulation, and a decrease in salinity are projected to be the direct consequences of climate change on marine creatures over the next century (Harvell et al., 2002). Additionally, atmospheric circulation changes will also influence precipitation patterns that in turn will affect turbidity, and inputs of terrestrial-derived nutrients and pollutants (Pisias et al., 2001). As a result of the consequent stratification of the water column, the decreased water mixing, and a deepening of the thermocline could prevent cool, nutrient-rich waters from being upwelled; therefore, nutrient availability and primary production will be impacted (Hughes, 2000; Roemmich and McGowan, 1995). This could alter the food web dynamics, decrease the abundance of species that construct habitats, shift species distributions, and increase disease incidence (Hoegh-Guldberg et al., 2010). In addition, storm systems may become more intense, and the hydrological cycle may undergo other changes as a result of ocean warming. For instance, altering air circulation is related to altering storm frequency (Bromirski et al., 2003); an increase in the frequency of winter storms has already been observed in coastal oceans, and the trend is expected to continue (IPCC, 2001).

1.3.1 Sedimentation

Light availability is one abiotic factor negatively correlated with diversity over depth range. In fact, below 10 m the decrease in light intensity is much more gradual. Thus, the total energy available for photosynthesis is also reduced with depth, although the presence of photosynthetic algae and coral at depths of 60 m or more, indicates that even extremely low light levels are sufficient to support some growth (Huston, 1985). This relates to the fact that coastal coral reefs are exposed to increasing amounts of nutrients, sediments, and pollutants washing off cleared, fertilisers, and urbanized catchments (Spalding et al., 2001) that reduce light availability, threatening their survivorship. Sediment suspension is in fact a stressor for coral reefs. The action of wind and rain, transport the eroded soil resulting from activities such as farming, building or road construction, deforestation, and dredging. The burying of living corals, tissue necrosis, abrasion, shading, and a population boom of bacteria in coral mucus are among the most significant effects of sediment stress. Furthermore, the amount of light in the water column is decreased by suspended particles, which has an impact on how quickly coral grows (Hubbard and Scaturo, 1985; Erftemeijer et al., 2012; Hughes et al., 2017). Different coral species react differently to the presence of sediments according to different characteristics such as their growth form and the size of their polyp (Erftemeijer et al., 2012). Elevated sediment deposition can lead to coral mortality and, afterwards, corals can be replaced by algae, which inhibits coral recruitment and thus has negative consequences for reef recovery (McCook and Diaz-Pulido, 2001).

Corals adopt a variety of mechanisms to keep the colony surface sediment-free, including passive rejection by branching morphologies or active rejection via polyp action, tissue expansion and contraction, and mucus production and shedding (Duckworth et al., 2017; Stafford-Smith and Ormond, 1992). Terrestrial runoff containing large amounts of suspended sediments and nutrients may enhance nutrient availability for benthic algae and phytoplankton altering their symbiotic relationship with zooxanthellae (Koop et al., 2001), it may reduce corals growth rate due to light reduction (Hunter and Evans, 1995), and it may reduce coral sperm availability (Ricardo et al., 2016), alongside larval settling (Fabricius, 2005; Perez et al., 2014). According to Nicholls et al. (2007), there is growing concern that the rates of human-caused losses in coastal water quality could be faster than the ability of delicate yet significant marine ecosystems like coral reefs to adapt. The energy required for coral survival in turbid waters is high (e.g., mucus production for sediment removal) (Brown and Bythell, 2005), which means less energy will be available for other energetic needs like growth and reproduction (Tomascik and Sander, 1985; Riegl and Branch, 1995; Fabricius, 2005)

1.3.2 Ocean Acidification

The ocean carbonate system is impacted by CO_2 addition, either passively or actively, which changes pH and carbonate-ion concentration (Zeebe and Wolfgladrow, 2001). The ocean plays an important role in reducing atmospheric CO_2 by absorbing about a quarter of CO_2 released each year into the atmosphere (Sabine et al., 2004; Reid et al., 2009). This absorption of anthropogenic CO_2 causes the acidification of the surface layers of the ocean, with a decrease of 0.02 pH units per decade over the past 30 years and an overall decrease since the pre-industrial period of 0.1 pH units (Harvell et al., 2002). Indeed, under a high CO_2 emission scenario, it is anticipated that ocean pH will decrease by an extra 0.3 pH units by 2100 (Bindoff et al., 2019), with carbonate saturation levels potentially falling below those required to sustain coral reef accretion; therefore, they could start dissolving themselves (Hoegh-Guldberg et al., 2007; Silverman et al., 2009). Furthermore, it is important to highlight that these changes have the potential to have strong impacts on marine biota (Kleypas et al., 1999; Seibel and Walsh, 2001; Kurihara et al., 2004).

Two processes, called pumps, are responsible for the uptake of carbon dioxide from the atmosphere to the ocean. The partial pressure of the gas on water increases together with the amount of CO_2 in the atmosphere. Most of the atmospheric CO_2 is absorbed by water (Gattuso et al., 2015). This is the so-called solubility pump. The second one is called biological pump. Primary producers at the bottom of the food chain in the ocean, capture CO_2 to make energy and organic matter, releasing oxygen as a byproduct (Gao et al., 2018). The carbonate system, through the biological pump, is the one controlling the pH of the water and the atmospheric CO_2 levels (Zeebe et al., 2021). Once transferred, carbon dioxide reacts with water molecules to form carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}). The result is an increase in the concentration of H^+ ions. This causes a decrease in pH, hence an increase in the acidity of the marine environment (Hoegh-Guldberg et al., 2007). The outcome is the slowing down of the aragonite deposition process and, in turn, the linear decrease in skeletal extension and density (Doney et al., 2009). Declining pH (increasing acidity) may affect other organisms in ways that extend beyond declining calcification or metabolic performance, including: alterations in interactions between species during different life stages, shifting competitive pressures (e.g., algae outcompeting corals), alterations in predation, and alteration of fish larvae behaviour (due to impaired sensory function in larval fish) leading to reduced recruitment success (Munday et al., 2010). Laboratory studies have examined the effects of ocean acidification on many types of corals and coralline algae, revealing a range of responses from a 3% to 60% decline in calcification rate for a doubling of atmospheric CO_2 (Kleypas et al., 2006).

1.3.3 Sea-Level Rise

Sea level will rise because of temperature rise. Raising a body of water's temperature, even slightly, results in an increase in its volume. If we think of the continents and the land as barriers, an ocean must increase in height. In addition, there is the issue of land-based glacier melting brought on by global warming. The volume of the sea rises as more water enters it (Gattuso et al., 2015). For instance, the Greenland ice sheet melting alone might result in a 7 m rise in sea level (Rignot et al., 2019). Whereas warmer waters have a shallower circulation, colder ones have a deeper circulation. The mixing characteristics of water masses, the rate of stratification, and the development of currents are all impacted by changes in ocean temperatures and volume (Gao et al., 2018). Current sea-level change is very slightly (10%) influenced by changes in terrestrial water storage as a result of natural climate variability and human activities (such as subterranean water mining, irrigation, urbanisation, and deforestation) (Milly et al., 2010). By contrast, intensive dam building along rivers during the second half of the 20th century lowered sea level by ~−0.5 mm/year (Chao et al., 2008). According to satellite altimetry, the rise in sea level is not constant. Since 1993, the sea level has risen up to three times faster than the world average in some areas, such as the western Pacific. Sea-level trends' spatial patterns are primarily caused by changes in salinity and non-uniform ocean warming (Change, 2007; Wunsch, 2007), although other factors also contribute, such as gravitational effects, and freshwater input (Milner et al., 2009; Stammer et al., 2008). Especially in coastal areas, sea level rise has a profound influence on the environment and society. Storm surges, erosion, and coastal floods all get worse and more frequent as sea level rises. This can harm coastal infrastructure, residences, and businesses, resulting in huge financial losses (Hallegatte et al., 2013). Moreover, sea level rise may have a big social effect. Millions of people could be forced to relocate due to it, especially in low-lying coastal communities where persistent flooding could result from sea level rise (McGranahan et al., 2007). Submergence, increased floods, and saltwater intrusion in surface waters are the direct effects on coastal land. As the coast adapts to the changing conditions, longer-term repercussions also take place, such as increased erosion and saltwater intrusion into groundwater. If coastal wetlands like salt marshes and mangroves lack the sediment supply needed to keep up with sea level rise, they will also suffer (Change et al., 2014).

1.3.4 Sea Temperature Rise

The ocean acts as a climate moderator in several ways: it absorbs much of the Earth's heat, compensating for the rise in the planet's temperature due to the greenhouse effect. Yet, this tendency causes the ocean's temperature to gradually rise. By 2100, the water temperature is projected to rise by 1-4 degrees from its current level (Dobson et al., 2021).

In comparison to the post-industrial age, the global temperature has been steadily rising over the past three decades (Gao et al., 2018). For the past 30 years, the rise in atmospheric greenhouse gas concentrations has resulted in an increase in global average temperatures of about 0.2 °C every decade, with the majority of this additional energy being absorbed by the oceans. As a result, since 1975, the heat content of the top 700 m of the world's ocean has increased by 14×10^{22} J, raising the average temperature of the upper layers by 0.6 °C over the previous century (IPCC, 2001).
90% of coral reefs might be lost if ocean temperatures rise by 1.5°C, and 90% could be lost if ocean temperatures rise by 2°C, according to the IPCC (Bindoff et al., 2019). In agreement with the presented scenario, tropical coral reefs could be completely lost in 2050 (Hoegh-Guldberg et al., 2010). Coral reef degradation may have a negative impact on nearby ecosystems, such as mangrove and seagrass systems that rely on reefs to protect them from wave action. Moreover, these climatic changes in salinity, acidity, and temperature are so rapid that may affect mangrove and seagrass distribution, sexual reproduction patterns, growth rates, and metabolism (Johnson et al., 1995; Short and Neckles, 1999), and ecosystems and living organisms would be unable to adjust to the new conditions leading coral reef ecosystems effectively and quickly, creating an algal-dominated habitat (Roth et al., 2021). Additionally, the dynamics of the population will be significantly impacted by changes in ocean circulation, which have a significant impact on larval travel. Also, it is important to evaluate the interactions between climate and other anthropogenic factors, particularly fishing pressure, which is likely to worsen climate-induced changes (Harley et al., 2006).

1.4 Study Area: Singapore

Singapore is an island country and city-state in maritime South-East Asia. It is located 137 km (one degree) north of the equator, off the southernmost tip of the Malay Peninsula, and is bordered to the west by the Strait of Malacca, to the south by the Singapore Strait, to the east by the South China Sea, and to the north by the Straits of Johor. With a total area of 734.3 km^2 and a population of around 5.6 million, Singapore is one of the most densely populated countries in the world (singstat.gov.sg, 2016). The nation's territory is made up of 63 satellite islands and islets in addition to one major island called Pulau Ujong (Savage et al., 2003). Moreover, it lies at the edge of the Indo-Pacific Coral Triangle region. Singapore has a tropical rainforest climate with no distinctive seasons, uniform temperature and pressure, high humidity, and abundant rainfall (Hess and Tasa, 2011; weather.gov.sg, 2016). Temperatures usually range from 23 to 32 °C. Singapore's climate can be divided into three periods: wet (average rainfall 300 mm $month^{-1}$) northeast (NE) monsoon period (November–March), drier (average rainfall 150 mm $month^{-1}$) southwest (SW) monsoon (May– September) and the inter-monsoon (IM) periods (April and October) (Tanzil et al., 2016).

Prior to Sir Stamford Raffles' establishment in 1819, Singapore was a tiny island blanketed by forests, with only 150 native Malays living there (Hilton and Manning, 1995). In 200 years, the population increased reaching 5.638 million people by 2018. Continuous land reclamation works have been carried out since 1891. The city-territory state's rose from 523 km^2 to 734.3 km^2 in December 2022, representing a 38.5% increase in area, with the goal of transforming a tiny fishing village into a large commerce hub (Glaser et al., 1991; singstat.gov.sg, 2023). Singapore's population growth and economic progress have come at a heavy price. Substantial loss and degradation of Singapore's coral reefs have been documented (Chou, 2006). Much of the intertidal ecosystems, reef flats, and mangroves have been replaced since the early 1960s by port infrastructure, petrochemical companies, military, and recreational facilities, and covered with solid fills as seen in Fig. 8 (Hilton and Manning, 1995). Singapore's remaining 13.25 km^2 of coral reefs now largely occur as fringing and patch reefs around the southern islands and co-exist alongside industries, busy ports, and shipping channels (Burke and Selig, 2002; Tun, 2012). For this reason, nowadays corals are restricted to about 8 m depth and are represented by stress-tolerant taxa normally found in turbid waters characterised by high sedimentation rate (Dikou and Van Woesik, 2006; Guest et al., 2016) and severe light attenuation.

Fig. 1.7: Aerial map of Singapore comparison between 1984 (left) and 2020 (right) (https://earthengine.google.com/timelapse/#)

Moreover, underwater visibility has decreased from 10 m in the 1960s to 2 m or less today, and sedimentation rates have increased from 3-6 mg cm^{-2} day^{-1} in 1979 up to 5-45 mg cm^{-2} day^{-1} in more recent years (Low and Chou, 1994; Dikou and van Woesik, 2006; Erftemeijer et al., 2012; Tun, 2012). At the same time, the ongoing rise in sea level is likely to impact corals living at depth in the light-limited environments of turbid reefs (Chou, 2006). Consequently, mean coral cover significantly declines with depth, reaching less than 10% beyond the first 3 m below chart datum, demonstrating that decreased light availability is the main cause of losses at the deeper reef sections (Guest et al., 2016b; Chow et al., 2019). Live coral cover at these deeper depths (~6 m)

has also dropped from up to 45% in the 1980s to <5–16% today, with no recovery to historical levels. By comparison, on shallower reef zones (∼2–4 m depths at mid-tide), mean live coral cover ranges from ∼25–49% (Guest et al. 2016), with recent fluctuations in percent live cover that coincide after mass bleaching events (e.g., in 1998, 2010 and 2016). According to extensive research conducted over the past three decades, few coral colonies are only able to survive at the shallowest 6 to 8 meters of water due to extremely turbid conditions and little light penetration (Guest et al., 2016; Souter et al., 2021). Beyond this depth, sand and rubble take over and the living coral cover substantially decreases (Chou, 2006). Singapore has historically recorded 255 species of scleractinian coral, which accounts for around 30% of the world's diversity (Huang et al., 2009; Veron, 2000). Due to various anthropogenic stresses affecting local reefs, with extensive reclamation, dredging and other coastal developments (Chou, 1988a; Hilton and Manning, 1995; Poquita-Du et al., 2019), only 138‑160 species have been recorded more recently (Huang et al., 2009; Wong et al., 2018).

1.5 Studied Species: *Pachyseris speciosa*

Pachyseris speciosa is a zooxanthellate coral, and it is one of the most common and abundant corals found in the Central Indo-Pacific region with a wide distribution through various depths (DeVantier and Turak, 2017; Bongaerts et al., 2021). It is one of the most common corals dominating the shallow reefs here (Wong et al., 2018; Chow et al., 2019) and it is usually associated with *Cladocopium* and *Durusdinium* (Tanzil et al., 2016; Jain et al., 2020; Smith et al., 2020), two different genera of *Symbiodiniacae*. Its ecologically opportunistic nature is reflected in being one of the most common species throughout Singapore's reefs (Fig. 1.8) (Guest et al. 2016; Wong et al. 2018) and across depths (Chow et al. 2019). Ship movement rousing the seafloor of the Singapore Strait (Browne et al. 2014, 2015) and the extensive land reclamation and development projects are the main causes of the turbid water in the reefs of Singapore (Dikou and van Woesik 2006; Sin et al. 2016). Due to this high turbidity and the consequent high light attenuation (i.e. < 1% of surface PAR at ∼9 m depth; Todd et al. 2004), laminar corals with large areal extents for light capture, such as *P. speciosa*, are most common at 3–8 m depth (Dikou and van Woesik, 2006; Guest et al. 2016; Chow et al. 2019). In fact, *P. speciosa* responded rapidly to decreased light availability by increasing the photosynthetic potential, or maximum quantum yield of photosystem II (Fv'/Fm') (Browne et al., 2014). As a result, these corals are commonly found within inshore turbid waters, and in Singapore are typically found in high abundance (>5%) on the upper reef slopes (<4 m LAT; Dikou & van Woesik 2006), suggesting adaptation to low-light, high sediment waters (Browne et al., 2015).

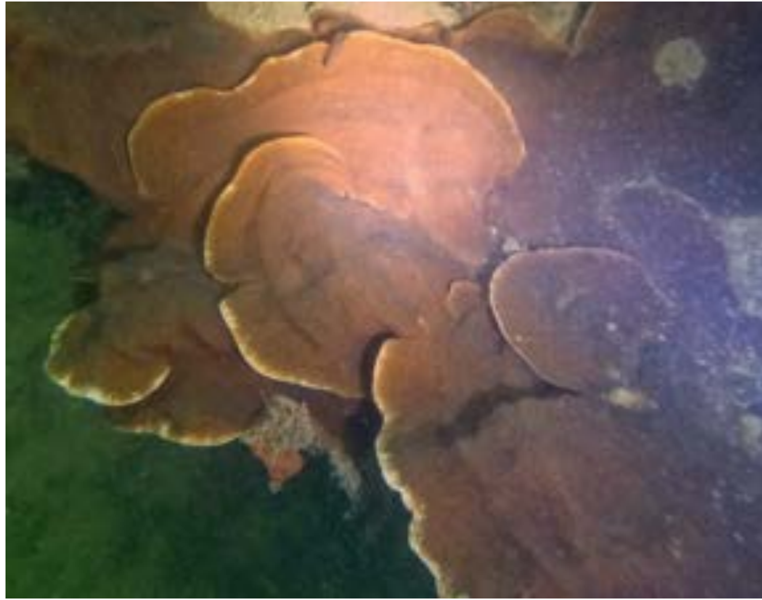


Fig. 1.8: Wild *Pachyseris speciosa* colony in Pulau Hantu, Singapore, ~7 m (Lorenzo Massimo Toniolo)

Reef-building corals, like *P. speciosa*, typically adapt to low light conditions by lowering their energy needs by reducing their tissue biomass, skeleton thickness, respiration rates, translocation, and growth (Anthony and Hoegh-Guldberg, 2003). *Symbiodinium* algae increases photosynthetic pigments and photosynthetic efficiency in low light acclimated corals to enhance light absorption and use (Falkowski and Dubinsky, 1981; Anthony and Hoegh-Guldberg, 2003). Interestingly, laminar corals, such as *P. speciosa*, develop funnel shapes, which reduce the surface area impacted by sedimentation by channelling and concentrating sediments to the colony's centre sections (Sofonia and Anthony, 2008).

1.6 Photo-Physiology

One of the main factors promoting the growth of marine photoautotrophs and symbiotic organisms is light energy. The marine environment, which creates a very dynamic environment, has a significant impact on the ability of photosynthetic organisms to capture light and produce energy (Krause and Weis, 1991). Pulse Amplitude Modulated (PAM) is a rapid, nonintrusive, non-destructive, real-time measurement of photosynthetic characteristics of marine and freshwater organisms (Kolber and Falkowski, 1993), in this case was used for corals. It measures the photosynthetic efficiency of photosystem II (PSII) within the endosymbiotic *Symbiodinium spp*. (Warner et al. 1999). The instrument involves a spectrofluorometric measurement of the kinetics of fluorescence rise and decay in the light-harvesting antenna of thylakoid membranes, thus querying various aspects of the state of the photosystems under different environmental conditions (Schreiber, 2004). In addition, PAM devices enable measurements in the presence of ambient light, in contrast to the conventional dark-adapted chlorophyll fluorescence observations.

Active fluorescence methods, such as the DPAM used in this study, have the advantage of being deployable in situ and delivering results in real time (Bhagooli et al., 2021). The principle underlying chlorophyll fluorescence analysis is relatively straightforward. Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates (Fig. 1.9):

- Can be used to drive photosynthesis (P, photochemistry)
- Can be dissipated as heat
- Can be re-emitted as light-chlorophyll fluorescence (Fl)

These three processes are in direct competition with one another, thus any improvement in one's efficiency will lead to a reduction in the yield of the other two. Therefore, information regarding changes in the effectiveness of photochemistry and heat dissipation can be obtained by measuring the yield of chlorophyll fluorescence (Maxwell and Johnson, 2000). PAM fluorometry is based on the quenching analysis (Misra et al., 2012), meaning that the sample is pulsed (at specific intervals) with a continuous high-intensity light at high frequency and the fluorescence emitted from the sample is detected. This offers a quick and effective method for detecting fluorescence emission when background measuring light is present (Schreiber et al., 1986; Schreiber, 2004; Misra et al., 2012). Photo-physiology can also be used as a parameter for photoacclimation. Typically, photoacclimation occurs when the PSII photoinactivation diminishes under the influence of a stressor, and Fv/Fm values initially decline before stabilising at a new level. On the other hand, persistently high Fv/Fm values, which are frequently a sign of low energy acquisition and can result in reduced growth and/or reproduction, mortality, or bleaching, for instance in hard corals, can be a sign of chronic photoinhibition at the level of PSII (Cantin et al., 2007).

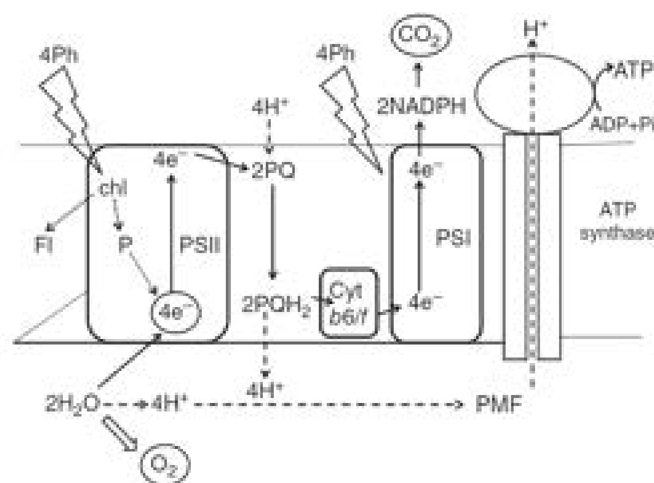


Fig. 1.9: Scheme of photosynthetic mechanism. Full arrows represent electron flow, the broken arrow proton flow, and the dotted arrows on the left the division of energy between photosynthesis (P) and fluorescence (Fl) during de-excitation of chlorophyll (chl) excited by photons (Ph) (Beer et al., 2014)

1.6.1 Quantum Yield

In photochemical processes, it is the parameter that has proven to be most useful is the effective quantum yield (light-adapted) of PSII, ΦPSII, measured with a saturating light pulse (Khoo and Mazlan, 2013) and it determines the efficiency of PSII photochemistry. It gives a measure of how much light is absorbed by the chlorophyll molecules connected to the PSII, or in photochemistry (Maxwell and Johnson, 2000). The molar ratio of oxygen evolved, or carbon dioxide absorbed to photons absorbed during photosynthesis, or the quantum yield of the PSII, thus gives an informative measure of photosynthesis (Ralph et al., 2002). Effective Quantum Yield (EQY) is determined from the light-adapted corals (Genty et al., 1989). The maximum capacity of PSII is represented by its highest photochemical efficiency. After dark acclimation, this parameter, also known as Maximal Quantum Yield (MQY), is assessed (Kitajima and Butler, 1975; Ralph et al., 2002). For instance, in marine invertebrates and plants, chlorophyll fluorescence, and photosynthetic performance can be used as a tool to evaluate stress conditions, stress tolerance, and the possibility of acclimatisation and/or adaptation (Bhagooli et al., 2021).

1.6.2 Other Photosynthesis Parameters

Maximum Electron Transport Rate (ETR_{max}) indicates the assimilatory power, which represents the initial products (NADPH and ATP) of the conversion of light energy into chemical energy (Hall and Rao, 1999). It entails both the assimilatory and non-assimilatory electron flow, which thus can be taken as electron flow beyond PSII (Bhagooli and Hidaka, 2006) and it can be used as an indicator of photosynthetic capacity (Figueroa et al. 2003). It considers the amount of light reaching the coral differently from $rETR_{max}$.

The term E_k is the minimum saturation irradiance parameter (sometimes also named Ik) and describes the transition between light-limited and light-saturated photochemical efficiency (Hennige et al., 2008), it is indicative of actual photochemical capacity (Suggett et al., 2010) and of photoacclimation (Hennige et al., 2008); it usually follows a simple linear relation with irradiance levels (Suggett et al., 2012).

Alpha (α) is the photochemical efficiency of photosynthesis, it increases with optical depth to increase light harvesting under low light conditions (Perkins et al. 2006). Alpha is obtained as a line tangential to the initial portion of the P–I curve (Beer et al., 2014).

1.7 Transcriptomic

Transcriptomic studies have emerged as a powerful tool to investigate how corals respond to a range of environmental conditions, including heat stress, acidification, bacterial challenge, turbidity, and deoxygenation (Alderdice et al., 2021; Cziesielski et al., 2019; Poquita-Du et al., 2019;

Strader et al., 2020; Wright et al., 2017); additionally, they have helped identify molecular traits that contribute to stress tolerance (Barshis et al., 2013). They have changed the way coral scientists approach important biological questions as they provide information on the entire suite of transcripts that are regulated across different environmental conditions. This method is very helpful for examining organisms' physiological states and their ability to survive under various stress levels (Evans and Hofmann, 2012).

At the molecular level, when exposed to heat or other stressors, coral hosts respond with extensive gene expression throughout a variety of metabolic pathways (DeSalvo et al., 2008; Meyer et al., 2009; Polato et al., 2010; Bellantuono et al., 2012; Barshis et al., 2013). Moreover, transcriptome-based approaches are beginning to uncover the underlying mechanisms by which endosymbiotic dinoflagellates withstand stressful conditions. According to the kind of *Symbiodiniaceae* harboured, it was discovered that the expression of *Symbiodiniaceae* genes connected to temperature tolerance and photosynthesis (i.e., chloroplast membrane components) varied during experimental procedures (Barshis et al., 2014).

RNA sequencing, or RNA-Seq, is a relatively recent high-throughput sequencing approach that generates data for transcriptome-wide profiling suitable for non-model organisms such as corals (Kenkel and Matz, 2016). Coral host and symbiont genetic and transcriptome data are readily available, enabling independent analyses of these distinct parts. Transcriptome-wide profiling, which provides details on the whole set of transcripts that are regulated across various environmental circumstances, has altered how coral scientists address significant biological concerns (Poquita-Du et al., 2019). This method is very helpful for examining an organism's physiological state and ability to survive under various stress levels (Evans and Hofmann, 2012).

2. AIM OF THE STUDY

2.1 Rational

Singapore's reefs suffer from high sedimentation and low light penetration as a result of extensive land reclamation coastal development since the 1960s. Underwater visibility in the Singapore Strait has reduced from >10m in the early 1960s to ∼2m today. Average daily photosynthetically active radiation (PAR) levels at ∼3 m fluctuate from a maximum in March/April (>500 µmol m^{-2} day^{-1}) and October/November, to as low as ∼50–80 µmol m^{-2} day^{-1} in June/July/August and December/January (Tanzil et al., 2019). With a light attenuation (K_d) of ∼0.3–0.5 (Tun et al., 1994), light levels at 6 m depths can therefore reach mesophotic levels which can be further exacerbated by coastal development activities that result in acute and extreme low light conditions. Despite such an environment, corals still persist on Singapore's turbid reefs. Understanding their photo-physiological responses in such conditions may provide insight into how corals can survive on urban marginal reefs.

2.2 Aim and Objectives

This study aims to understand the photo-physiological responses of the coral *P. speciosa* subject to three different light conditions: 1) Control (C): average light conditions on Singapore's reefs at ∼3 m, 2) Intermittent Light/dark (IL) treatment simulating possible scenario where corals are subject to acute low/no light as a result of coastal activities (e.g., dredging, land reclamation) or extreme coastal runoff event (e.g., after intense storms), 3) complete darkness (D) mimicking the possible scenario of extended unmanaged coastal development activities or sedimentation events. Additionally, we aim to understand if *P. speciosa* is able to quickly adapt to abnormal light conditions both at a photo-physiological and transcriptomic level. The obtained results could therefore be useful to advise governmental institutions in explaining how corals respond to acute low-light, and which mechanisms allow Singapore's corals to withstand the worsening of water conditions.

To achieve these goals, during this thesis experiment, *P. speciosa* was subjected to an acute low-light stress experiment to be lasted four weeks under three different treatments: Control (actual conditions), Intermittent Light (pulse disturbance conditions) and Dark (extended bad conditions). To obtain information about the coral stress response and health condition, coral samples were subjected to different treatments and then analysed by using three approaches: 1) Bleaching status. Zooxanthellae density, chlorophyll a concentration, and colour score were used as indicators. 2) Photo-physiology. It was investigated using a PAM fluorometer, and the following parameters were measured: Rapid Light Curves (RLCs), Maximum Quantum Yield (MQY, Fv'/Fm'),

Effective Quantum Yield (EQY, Fv/Fm) which are indicators of photosynthetic performance. Fitting the RLCs data we also obtained: Electron Transport Rate max (ETR_{max}), minimum saturation irradiance (E_k), and photochemical efficiency (Alpha). 3) Transcriptomic. RNA-seq helped to assess the stress level of the coral host at a molecular level analysing the gene expression patterns.

3. MATERIALS AND METHODS

3.1 Experimental Design

3.1.1 Coral Sampling and Fragmentation

For the experiment, four different parental colonies of *Pachyseris speciosa* were provided by St. John's Island Marine Laboratory, Singapore. They were collected around Kusu Island (Fig. 3.1) in March 2023 and then cultured in SJINML aquaria under artificial lights and with water directly coming from the sea around St. John's Island.

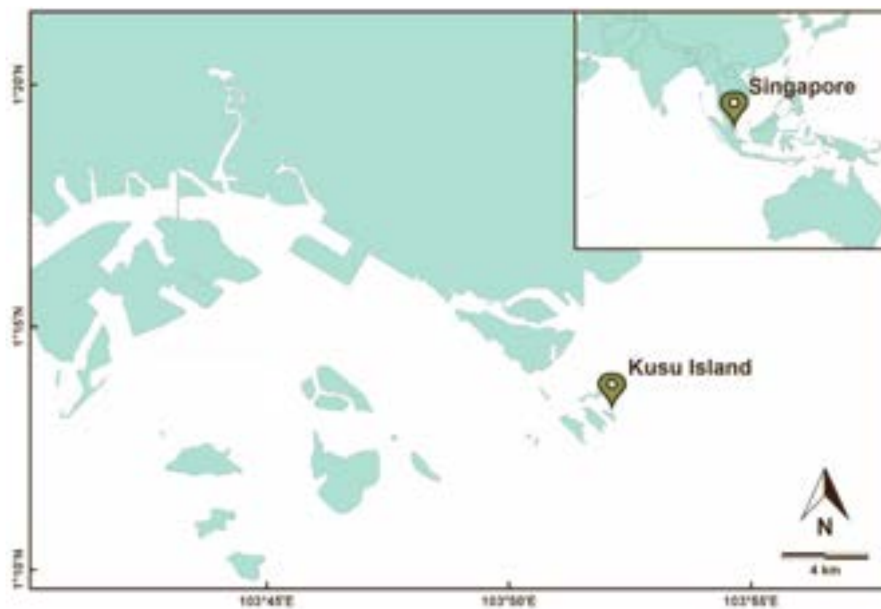


Fig. 3.1: Collection site (Adapted from Maggioni et al., 2022)

Each parent colony was cut in nubbins of ~4 cm^2. The number of fragments obtained was based on the colony size. The exact date of fragmentation was the 28th of April 2023. In total, 68 nubbins were obtained from the four *P. speciosa* parental colonies. In particular, 17 fragments were obtained from the parental colony marked as PCHS 10, 19 fragments from PCHS 19, 17 fragments from PCHS 18, and 18 fragments from PCHS 11. Corals were fragmented manually using a Rotatory Tool Kit (Dremel 8200) equipped with a cut-off wheel. Subsequently, they were attached to Lego® cubes (prepared in advance and using different colours to differentiate the parental colonies), using Premium Aquatic Glue (Aquatic Exclusive). All the Legos® were previously labelled with a specific barcode and acronym (e.g. PCHS 10.18). PCHS stands for the genus name (*Pachyseris*); the first number stands for the parental colony (10); finally, the second number refers to the fragment number (18). After fragmentation, corals were kept in an aquarium to acclimate for seven days before starting the experiment. After one week of acclimation, corals were further fragmented. From each fragment, two sub-fragments were collected. Specifically, one of them was then used for transcriptomic analysis, whereas the second one for photo-physiological analysis. Fragments

used for photo-physiology were immediately frozen at -80 °C, whereas fragments for transcriptomics were put in RNAlater, left in a normal fridge at 4 °C for 24h and then frozen at -80 °C. After this fragmentation, corals were left acclimating for 36 hours before the actual start of the experiment.

3.1.2 Aquarium Set-Up

Coral's fragments were put in a big tank of 150 x 110 x 70 cm in size, in order to let them acclimate. This tank is located outdoors, and it is uncovered. At the same time, 12 PVC tanks, completely transparent of 36 x 22 x 22 cm in size, were put in the same big tank on the 3rd of May 2023 with water flowing singularly inside each of them to let any unwanted particles flow away prior the start of the experiment. A pipeline system *ad hoc* was designed and built. Specifically, it aimed to create a water flow going from the very bottom of the plastic tanks up to the surface. Doing so, the water overflows from the top of the tank (Fig. 3.2). Moreover, being the plastic tanks for a little part above the water level (the water level was kept constant at 20 cm in height in the Green Tank), this ensured a complete closeness of the system in respect to all the other tanks; the experimental system can be considered isolated. The pipeline system was built using PVC pipes and connectors (elbows, Ts, etc.). It was connected to the main pipeline system taking water directly from the sea. The system was built to minimize any pipeline shade on the coral's fragments. Moreover, the final part of the pipes (the one going directly into the tank) was placed at one of the two corners of each PVC tank.

Since the corals were directly receiving water from the ocean, they lived under the following conditions:

- Light: since the facility was located outdoor, two shading layers were applied over the Green Tank, resulting in a lowering of the PAR from 850 µmol/(m²·s) to 220 µmol/(m²·s) (calculated with an Apogee, model MQ-510)
- Temperature: since the incoming water temperature was between 29-32 °C, a chiller (HAILEA, model HK-1000A) helped to maintain it constant at 29 °C
- Nutrients: being directly in contact with the ocean, corals didn't need any additional nutrients as they were provided and contained in the water itself
- Sedimentation: Seawater coming into the aquarium facility is sand-filtered down to ~200 µm

Fig. 3.2: Experimental set-up (photo: Lorenzo Massimo Toniolo)

The shading system around the tanks was built using black plastic corrugated boards, 3mm thickness, paying attention not to leave any undesired light income taping all the fissures with 3M tape. The sheets were then screwed together using aluminium angle bars, bolts, and nuts, forming boxes. Only a little hole in one corner on the top cover was left open to allow just the pipe to get directly into the tank, allowing for continuous water circulation. The top part of the box was attached with Velcro® to the sides of the box shading system. This was done to facilitate the removal of the top part of the shading during maintenance operations and for Intermittent Light treatments (Fig. 3.3).

Fig. 3.3: Shading system built with plastic corrugated boards and 3M tape (Lorenzo Massimo Toniolo)

Corals were subjected to three different treatments:

- Control (C): corals followed the natural day-night cycle mimicking their normal niche conditions (~35 µmol m^{-2} s^{-1} that can be found at 6-7m depth)
- Intermittent Light (IL): corals were left in complete darkness for five days a week, and every Tuesday and Friday were unshaded for an entire day for normal light conditions (between 9:00 and 16:30)
- Dark (D): corals were completely covered for the entire period of time

To minimise any bias regarding the shading system, a box without the top cover part was built also for Control treatments (mimicking the Intermittent Light treatment when unshaded). At the beginning, the experimental design also included another species, *Goniastrea pectinata* (Fig. 3.2 and 3.4). However, during the experiment some technical issues resulted in tank setups housing the *Goniastrea pectinate* corals being compromised, and it was therefore decided to exclude it from the project. Fortunately, as the two study species were kept in separate tanks, the experiment could proceed with the remaining study species, *Pachyseris speciosa*.

The tanks were placed in two rows, so, six tanks per row. In every row, the two coral species were alternated (PCHS - GP - PCHS - GP - PCHS - GP). The position of the different treatments in the tank were completely randomized, in order to avoid any bias (Fig. 3.4).

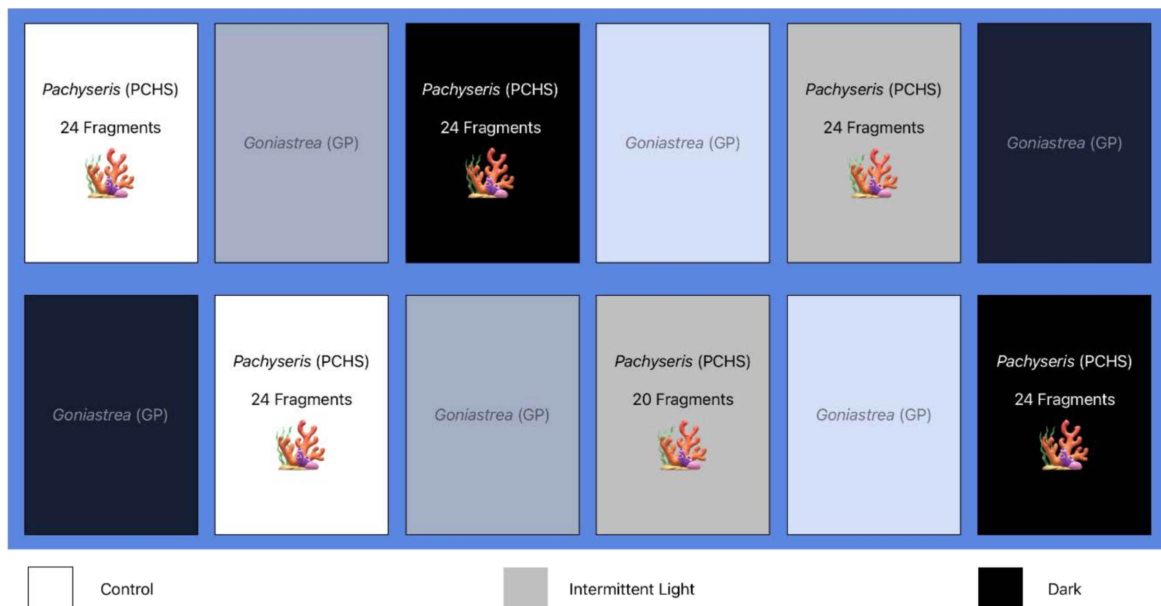


Fig. 3.4: Experimental set-up, the total number of fragments is 68. In each tank 12 fragments coming from four different parental colonies are present except in one of them where 10 fragments from 4 different parental colonies are present. *Goniastrea* (GP) should not be considered.

The tanks were numerated starting from the top left and continuing to the right. Therefore, the first row started with one, whereas the second with the number seven (from the left side). At the

beginning of the experiment, coral fragments were randomized in each tank, and they were put in order as follows: *Pachyseris* genera: 10 (red Logo® block) – 19 (yellow Logo® block) – 18 (grey Logo® block) – 11 (white Logo® block). In every tank, three fragments from genus 10, three fragments for genus 19, three fragments from genus 18, and three fragments from genus 11 were attached to Lego® tables, keeping the order consistent in every tank (Fig. 3.5). HOBO Data Logging Solutions model MX2202 were put in each tank to have a precise measurement of light and temperature every 10 min. Data were recorded continuously starting from 5:00 pm on the 15th of May 2023 to 11:50 pm on the 7th of June 2023. When analysed, mean light data were calculated between 7:00 am and 7:00 pm (from sunrise to sunset), following previous studies (Chow et al., 2019; Morgan et al., 2020). All the loggers were calibrated using a Light Sensor Logger LI-COR model LI-1500 (Fig. 8.1). The data collected through it had as a unit measurement lux. Therefore, they were converted in PPFD (PAR) by multiplying the LI-COR data times 0.0185 (https://www.apogeeinstruments.com/conversion-ppfd-to-lux/).

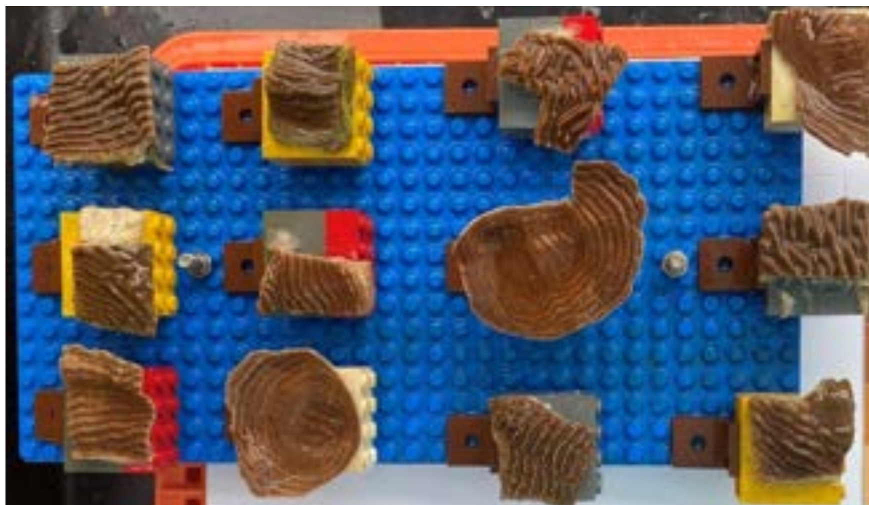


Fig. 3.5: Coral fragments glued on Legos® (Lorenzo Massimo Toniolo)

3.2 Data Collection

3.2.1 Colour Score

Coral colour score was assessed two times per week, for a total of eight times throughout the experiment. Coral plates were taken out of the water for a maximum of 30 s and put on a white plate with Coral Watch Chart colours (Fig. 3.6). Photos were taken trying to avoid any external light that could affect coral colours using an umbrella to shade sunlight. For all the assessments (except the 26th of May) photos were taken with an iPhone 11 Pro Max with standard settings and trying to maintain the same distance from the plate. Fragments' colour was assessed giving a number between one and six, where one represents a completely bleached coral, whereas six gives an

indication of a dark-coloured coral. This was done through visual assessment on a computer using the pictures taken.

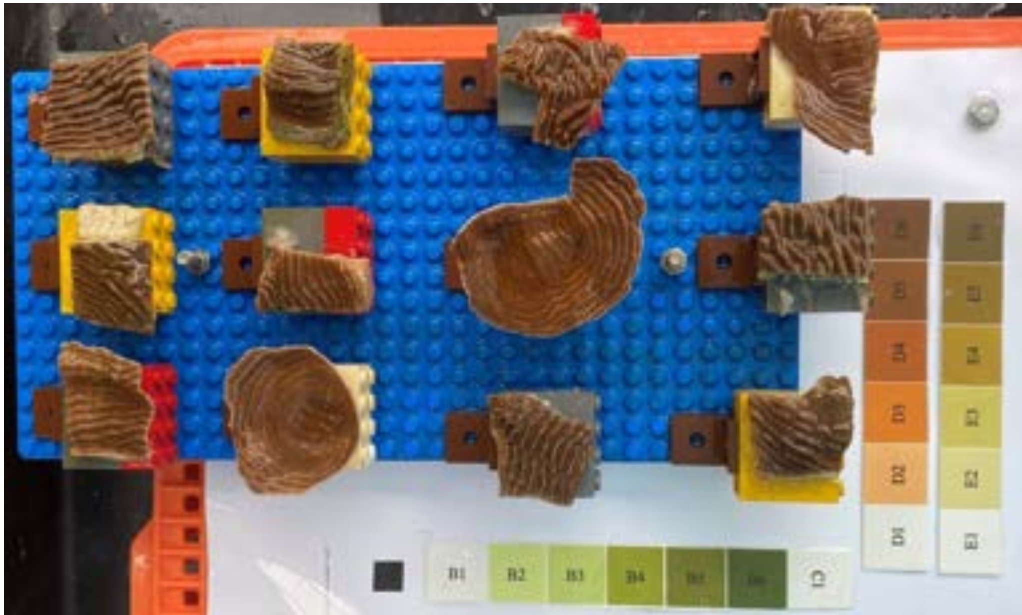


Fig. 3.6: Colour photo-evaluation of coral fragments with Coral Chart Colours (Lorenzo Massimo Toniolo)

3.2.2 Zooxanthellae Density

All coral nubbins (n = 68) were sub-fragmented 2 times, at the beginning and at the end, obtaining 1cm x 1cm fragments that were used for zooxanthellae density assessment. The density was obtained by normalizing the number of cells to the surface area of each fragment analysed. Corals were cut with a Dremel 8200 and immediately frozen at -80 °C to preserve them until the start of the analysis.

Zooxanthellae separation and count:

- Waterpiking:
    - Using clean forceps, Waterpik the coral fragment into a clean ziplock bag using a Waterpik waterflosser WP-660 UK. (Change the ziplock every 3 samples, using the same ziplock only for the same family's fragments)
    - Decant suspension and rinse with Milli-Q into a glass beaker. (Keep coral skeleton in their respectively labelled ziplock bag and store in a -20 °C freezer)
    - Homogenise the suspension for ~20s per tube with QIAGEN TissueRuptor
    - Rinse tissue ruptor for ~5s before 70% ethanol and after in Milli-Q before continuing
    - Measure the total volume of suspension in a graduate cylinder and record it in an Excel sheet

- Pipette and isolate 10ml from the suspension for zooxanthellae counting in a 15ml tube
- Transfer the remainder of the suspension and rinse with Milli-Q into clean 50ml tubes pre-labelled
- Balance all tubes with the weighing balance and centrifuge – 4,500 rpm for 15 minutes at 4°C (maximum weight difference = 0.2g). These will be used for chlorophyll analysis
- Discard the supernatant and keep the pellet. Merge in one tube and do again this process. Always weigh and balance all tubes with Milli-Q water

• Counting:
  - Clean the haemocytometer with 70% ethanol and Kimwipes from KIMTECH Science before use
  - Invert suspension (15ml tube) to ensure a homogenise suspension and pipette 10 µl to load into each side of the haemocytometer:
    - Clean the coverslip and place it on the haemocytometer
    - Pipette at a 45° angle to load the haemocytometer at the notch
    - Ensure no air bubbles are loaded before counting
  - Count all the 9 squares of the haemocytometer under a light microscope (Olympus) at 20x magnification. For cells along the boundary, only account for the top and left lying ones (Figure 3.7)
  - Repeat for 5 times using different aliquots, and fill the Excel sheet

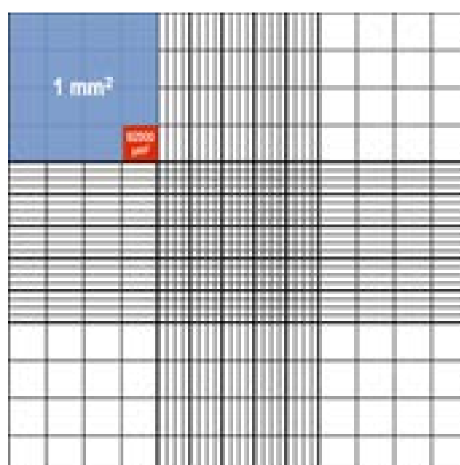


Fig. 3.7: Bürker's chamber

• Density:

  Using ImageJ the fragment surface area was calculated as follows:
  - Open the "Coral Calibration v1" file

- Set Min SD to 0.5 and SD increment to 0.1, then click ok
- Select the Image and open it
- Select the *straight* tool and measure one side of the 1 cm x 1 cm black square present in every picture
- Select "Analyze" and "Set Scale". Substitute in "Known distance" 1 cm
- Select the *freehand* tool. Using a mouse or a graphic tablet contour the entire coral perimeter as precisely as possible
- Select "Analyze" and "Measure", or just use the shortcut Ctrl + M. The result obtained is the fragment's surface
- Calculate zooxanthellae density the cell count in the total solution volume used for their extraction (cells/ml) divided by the fragment's surface area (cm^2)

3.2.3 Chlorophyll a Concentration

All coral nubbins (n = 68) were sub-fragmented 2 times, at the beginning and at the end, obtaining 1cm x 1cm fragments that were used for chlorophyll a concentration assessment at the beginning and at the end of the experiment. The concentration was obtained by normalizing the number of cells to the surface area of each fragment analysed. Corals were cut with a Dremel 8200 and immediately frozen at -80 °C to preserve them until the start of the analysis.

Chlorophyll a extraction:

- After having followed the "coral and zooxanthellae separation and count" centrifuging processes, continue here for chlorophyll extraction
- Decant supernatant and retain sample pellets. Try to dry it as much as you can, use a pipette. (Can be frozen in a normal freezer and processed later)
- Dose the desired amount of 99% ethanol (usually 8ml or multiples) into each sample tube and homogenise with the tissue ruptor
    - Rinse the tissue ruptor for ~5s before in Milli-Q and after in 99% ethanol between each sample
- Check if the colour is the desired one compared to the picture (Figure 3.8). The colour should be similar to the two middle cuvettes. If not, add more ethanol to dilute. Consequently, write on the tube the exact number of ethanol ml and fill the Excel sheet, it will be used to normalize the [chl a]

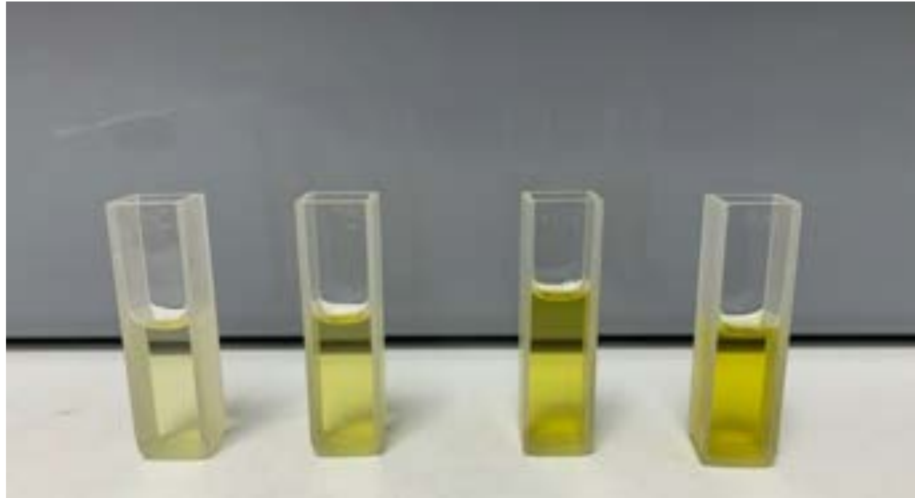


Fig. 3.8: Cuvettes with chlorophyll extract mixed with 99% ethanol. This should be the aimed range of colour of the extract

- Leave to extract for ~24 hours in the fridge
- Balance and centrifuge the samples at 4,500 rpm for 15 minutes at 4°C
- Set up the spectrophotometer to run a Photometric, Multicell analysis at 632nm, 649nm, 665nm and 696nm
    - "Connect" spectrophotometer after opening the UV Probe application
    - Select the "Photometric" option and go to "Method"
    - Add desired wavelengths (632nm, 649nm, 665nm and 696nm) and "Next"
    - Change Type to "Raw Data" and ensure "None" is selected for sample repetitions before "Finish"
    - Under "Attachments", "Initialise" multi-cell to the number of samples to be run at each time (maximum 6)
    - Key in the respective sample IDs into the table
    - "Blank" and "Autozero" after samples are loaded
- Rinse each cuvette 3 times with milli-Q and with 1 ml of 99% ethanol before use
- Pipette 3ml of reference blanks (99% ethanol) and extracted clear chlorophyll solution into each cuvette and load accordingly
- Wipe dry the smooth sides of the cuvette with Kimwipes and ensure the cuvettes are clean before loading
- Run analysis with "Read Unknown". Ensure the absorbance reading is between 0.1 - 0.5A, otherwise dilute the sample to get within range
- Take a picture of the results (sometimes the program can crash, and you can lose data). ONLY AFTER, discard samples into a designated glass waste beaker to be emptied into a waste container at the end of the session
- Save the data by selecting "Print" and saving the PDF report

3.2.4 Photo-Physiology

Photo-physiological data were collected using a Diving Pulse Amplitude Modulation Fluorometry (DPAM, Walz GmbH, Effeltrich, Germany) connected to a 6 mm diameter fiber-optic probe, and the following parameters were assessed:

- **Rapid Light Curves (RLCs)** - To assess photosynthetic activity. Since the position of the sun with its irradiance could have influenced the results, this was done in a separate indoor aquarium. Therefore, the corals were momentarily transported in a new tank with the same water parameters, to have a constant light irradiation since the process took approximately 4 hours. Moreover, having constant light was important since the process for every fragment took ~2 minutes, avoiding errors due to different PAR levels reaching the coral
- **Effective Quantum Yield (EQY)** - Shows how efficient a photochemical reaction is. It analyses the ratio of the number of photons emitted to the number of photons absorbed. The analysis was conducted after 13:00. It was performed directly in the plastic tanks, without changing coral conditions
- **Maximum Quantum Yield (MQY)** - Shows the maximum efficiency of the photosystem before the sun rises. It is the maximum number of events resulting from the minimum number of photons (quanta) absorbed. Since MQY requires dark adapted corals, it was performed before sunrise, at 4:30, directly in the plastic tanks, without changing coral conditions. However, only for Tank 10, MQY Beginning was done shading the corals at 17:00 on the 10th of May and collecting data on the 11th at 9:15 in a dark room

For optimal fluorescence acquirement, the parameter Transient Fluorescence (Ft) was kept between 120-140, with some exceptions under 120, but always above 100. DPAM settings were never changed within a sampling period (beginning or end), it was decided to be consistent instead of obtaining the desired Ft for all fragments through settings changes. However, settings had to be changed for the measurements at the end of the experiment. In fact, the same settings used at the beginning resulted non suitable for coral fragments after four weeks of treatment. Moreover, finding suitable settings both for Light, Intermittent Light, and Dark treatment resulted impossible, since corals lost zooxanthellae throughout the experiment. Therefore, Transient Fluorescence (Ft) wasn't kept at 120-140 for the end sampling period, it was instead decided to be consistent with DPAM settings to be able to compare the data collected.
Coral-*Symbiodiniaceae* photosynthetic performance was evaluated by measuring the maximum quantum yield (F_v'/F_m') and the effective quantum yield (F_v/F_m) of Photosystem II (PSII), where

Fm represents the maximum fluorescence with actinic light of the sample after a saturating pulse is applied, and Fv (Fm - F) is the variable fluorescence. Fv' and Fm' refers to the same things but in dark-adapted corals. Measurements were made at the start and at the end of the experiment on the coral tissue surface for each replicate.
DPAM settings beginning:

- Distance between clip and notch: 5.5cm
- Light settings:
  - Intensity: 1 (changed to 2 just for 3 samples)
  - Frequency: 1
- Gain: 1
- Damping: 1
- ETR-Factor: 0.84
- Program settings, light curve:
  - Width: 10 seconds
  - Intensity: 1
  - Length: 12 (from 1 to 13)

DPAM settings end:

- Distance between clip and notch: 5.5cm
- Light settings:
  - Intensity: 5 (changed to 2 just for 3 samples)
  - Frequency: 1
- Gain: 3
- Damping: 1
- ETR-Factor: 0.84
- Program settings, light curve:
  - Width: 10 seconds
  - Intensity: 1
  - Length: 12 (from 1 to 13)

A Miniature Spectrometer MINI-SPEC by WALZ recorded separately spectra of PAR in the visible and far-red range.
To fit RLCs curves, an R code written by Antti Takolander in September 2019, using the R package "phytotools" and the model by Platt et al. 1980 was used. Based on Bhagooli et al., 2021, five between the most used parameters have been used for the analysis: Maximum Quantum Yield (MQY, $\frac{Fv'}{Fm'}$), Effective Quantum Yield (EQY, $\frac{Fv}{Fm}$, ΦPSII), Maximum Electron Transport Rate (ETR_{max},

photosynthetic efficiency, $ps * \frac{\alpha}{\alpha+\beta} * (\frac{\beta}{\alpha+\beta})^{\frac{\beta}{\alpha}}$), Alpha (photochemical efficiency of photosynthesis at low light, the initial slope of the light response curve, $\frac{ETR}{PAR}$) and E_k (minimum saturation irradiance, $\frac{ETRmax}{\alpha}$). (β measures the decrease in photosynthetic efficiency due to photoinhibition).

3.2.5 Transcriptomics

Coral fragments (n = 24) were randomly selected to obtain a fragment of 1cm x 1cm, and to have four fragments per tank (therefore eight per treatment), both at the beginning and at the end of the experiment. Corals were cut with a Dremel 8200, cleaned after every nubbin fragmentation with ethanol 99% avoiding RNA contamination between fragments. After, they were put in pre-labelled tubes filled with RNAlater and kept in a normal fridge for 24h at 4 °C to allow RNAlater to penetrate in the sample, subsequently they were put in a -80°C freezer. The RNA extraction was performed using TRIzol Reagent provided by Thermo-Fisher according to the manufacturer's instructions.

Preparation of RNA samples:

- Prepare in 1.5ml microtubes 0.1g of 0.5mm Zirconia beds and autoclave them with all tubes needed (50ml and 1.5ml)
- Clean the fume hood bench thoroughly and repeatedly with 10% bleach and 70% ethanol
- Prepare the required materials (i.e. pipettes, racks, filtered tip boxes, reagents, parafilm pieces) and clean with 10% bleach before using. Only use designated pipettes for fume hood. Moreover, sterilise under UV light everything for 300s
- Aliquot required volumes of reagents in sterile tubes: TRIzol reagent, chloroform, 100% isopropanol, 70% ethanol (100% ethanol diluted in RNAse-free water), glycogen, RNase-free water **Note**: Always start with fresh chemicals. Aliquot to amount needed only
- Thaw tissue samples but keep them chilled in an ice box
- Dissect a small piece of tissue (∼50–100mg of soft tissue; ∼150–200mg of coral) from RNAlater-preserved sample and place them in 1.5ml microtubes pre-filled with Zirconia beads. Label tubes accordingly
- Add 1ml of TRIzol reagent directly into tubes and vortex for 10min. **Note**: Homogenisation is done at room temperature
- Centrifuge at 12,000rcf for 10min at 4°C to pellet waste tissue. Ensure the centrifuge has been set at required temperature before loading the samples and spinning. To adjust the temperature faster, select the 'fast temp' function and let it spin until it reaches the desired temperature

- Load the samples in the centrifuge. Ensure the centrifuge is well-balanced and the lid is properly closed
- Carefully transfer supernatant to pre-labelled fresh tubes by pipetting. Make sure not to include the beads and waste tissue
- Incubate for 5min at room temperature to permit complete dissociation of the nucleoprotein complex
- Add 200µl of chloroform (per 1ml of TRIzol reagent used for homogenisation) directly into the new tube and cap securely
- Shake the tube vigorously by hand for 15s and incubate for 2–3min at room temperature
- Centrifuge the samples at 12,000rcf for 15min at 4°C. Carefully retrieve samples from the centrifuge so as not to disturb the phase separation
- Transfer the supernatant by angling the tube at 45° and pipetting out the upper (aqueous) phase of the sample. Avoid drawing any of the interphase or organic layers into the pipette when removing the aqueous phase. Place the clear supernatant in a pre-labelled new tube. **Note**: Discard the remaining materials (i.e. interphase and organic bottom red layer) to the designated hazardous waste container
- Add 2µl of glycogen to each of the new tubes. Dip the pipette in the aqueous phase to ensure proper transfer of glycogen
- Add 500µl of 100% isopropanol (per 1ml of TRIzol reagent used for homogenisation) into the new tubes with the aqueous phase and glycogen
- Vortex to mix for 15s and incubate at room temperature for 10min
- Centrifuge at 12,000rcf for 10min at 4°C to pellet the RNA. **Note**: The RNA is often invisible prior to centrifugation, and will form a gel-like pellet on the side and bottom of the tube
- Decant (pour) the supernatant to the waste container, leaving only the RNA pellet
- Add 1.5ml of 70% ethanol and wash the pellet by flicking the tube sharply
- Vortex the samples for 15s and centrifuge the tubes at 7,500rcf for 5min at 4°C. Decant the wash to the waste container
- Perform another wash following the 2 last steps
- Carefully pipette out the remaining wash (if any) without drawing the RNA pellet. Air-dry the pellet for 5–10min. Do not dry the pellet by vacuum centrifuge. **Note**: Avoid drying the RNA completely because the pellet can lose solubility. Partially dissolved RNA samples have an A260/280 ratio <1.6
- Resuspend the RNA pellet in 40µl of RNase-free water and incubate in water bath or heat block set at 55–60°C for 15min (0 rpm)
- Proceed to downstream application (gel electrophoresis) to check the quality of RNA

- Store the sample at -80°C for further RNA sequencing

Electrophoresis protocol:

- Weight 0.7g of agarose, in order to make a 1% gel
- Pour 75ml of Milli-Q water into a bottle
- Mix the agarose and the water and put it in the microwave for 1 minute
- Carefully take out the bottle using a kitchen flap and let it rest for a couple of minutes. When warm, add some 2µl Gel Red Nucleic Acid and let it rest until it becomes warm
- Prepare the electrophoresis box with the 20 spaces cleaver
- Pour the solution into the chamber (still outside of the liquid) and let it rest for at least 30 minutes
- Cut some parafilm and sterilise it for 300 seconds under UV light, it will be used to mix the loading dye and the samples
- From the fridge take 1kb DNA Ladder (reference) and loading dye (6x)
- Aliquot on parafilm x 1µl loading dye droplets and mix them with 4µl of the RNA sample (do the same also for DNA Ladder) pipetting. When homogenised, put them in the electrophoresis gel carefully without piercing the gel
- Connect the two cables to the main part of the electrophoresis, set Voltage at 100 and minutes at 40. Then press start

3.3 Statistical Analysis

The Shapiro-Wilk test was used to assess the normality distribution of data. One-way ANOVA statistical test followed by Tukey's HSD post hoc tests for multiple pairwise comparisons of means were performed to assess significant differences between the different treatments tested. If the assumption of homogeneity of variances required by the one-way ANOVA was not met, the Kruskal-Wallis non-parametric statistical test was performed followed by pairwise comparisons. Pearson correlation coefficient was used to test associations between the measurements. All the data were analysed using IBM SPSS Statistics software, version 29.0.1.0.

4. RESULTS

4.1 Coral Bleaching Measurements

4.1.1 Zooxanthellae Density

Zooxanthellae density was measured at the beginning and at the end of the experiment for all 68 fragments of *P. speciosa*. Significant differences were detected between the different treatments analysed (one-way ANOVA, $F(5, 129) = 30.115$, $P < 0.01$, Fig. 4.1a).

For Control treatment no significant difference was observed between the start (C) and the end (C-E) of the experiment. Moreover, Intermittent Light treatment significant difference was observed between the start (IL) and the end (IL-E) of the experiment. There was a decrease of 32% ($P < 0.01$) decreasing from a mean value of $2.2 * 10^7 \pm 1.3 * 10^6$ cells cm^{-2} to a mean value of $1.5 * 10^7 \pm 9.7 * 10^5$ cells cm^{-2}. Finally, Dark treatment significant difference was observed between the start (D) and the end (D-E) of the experiment. There was a decrease of 70% ($P < 0.01$) decreasing from a mean value of $2.4 * 10^7 \pm 9.3 * 10^5$ cells cm^{-2} to a mean value of $7.3 * 10^6 \pm 7.2 * 10^5$ cells cm^{-2}.

4.1.2 Chlorophyll a Concentration

Chlorophyll a concentration was measured at the beginning and at the end of the experiment for all 68 fragments of *P. speciosa*. Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 56.840$, $p < 0.01$, Fig. 4.1b).

For Control treatment no significant difference was observed between the start (C) and the end (C-E) of the experiment. Moreover, Intermittent Light treatment significant difference was observed between the start (IL) and the end (IL-E) of the experiment. There was a decrease of 47% ($P < 0.01$) decreasing from a mean value of 22.5 ± 2.6 µg cm^{-2} to a mean value of 11.9 ± 2.2 µg cm^{-2}. Finally, Dark treatment significant difference was observed between the start (D) and the end (D-E) There was a decrease of 75% ($P < 0.01$) decreasing from a mean value of 26.4 ± 1.9 µg cm^{-2} to a mean value of 6.6 ± 0.9 µg cm^{-2}.

4.1.3 Colour Score

Colour score was measured twice a week for each of the 68 fragments of *P. speciosa* for a total of eight times per fragment. Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 80.072$, $p < 0.01$, Fig. 4.1c).

For Control treatment no significant difference was observed between the start (C) and the end (C-E). Moreover, Intermittent Light treatment significant difference was observed between the

start (IL) and the end (IL-E). There was a decrease of 12% (P < 0.05) decreasing from a mean value of 4.95 ± 0.12 to a mean value of 4.35 ± 0.16. Finally, Dark treatment significant difference was observed between the start (D) and the end (D-E). There was a decrease of 58% (P < 0.01) decreasing from a mean value of 5.42 ± 0.13 to a mean value of 2.29 ± 0.15 (Fig. 4.2).

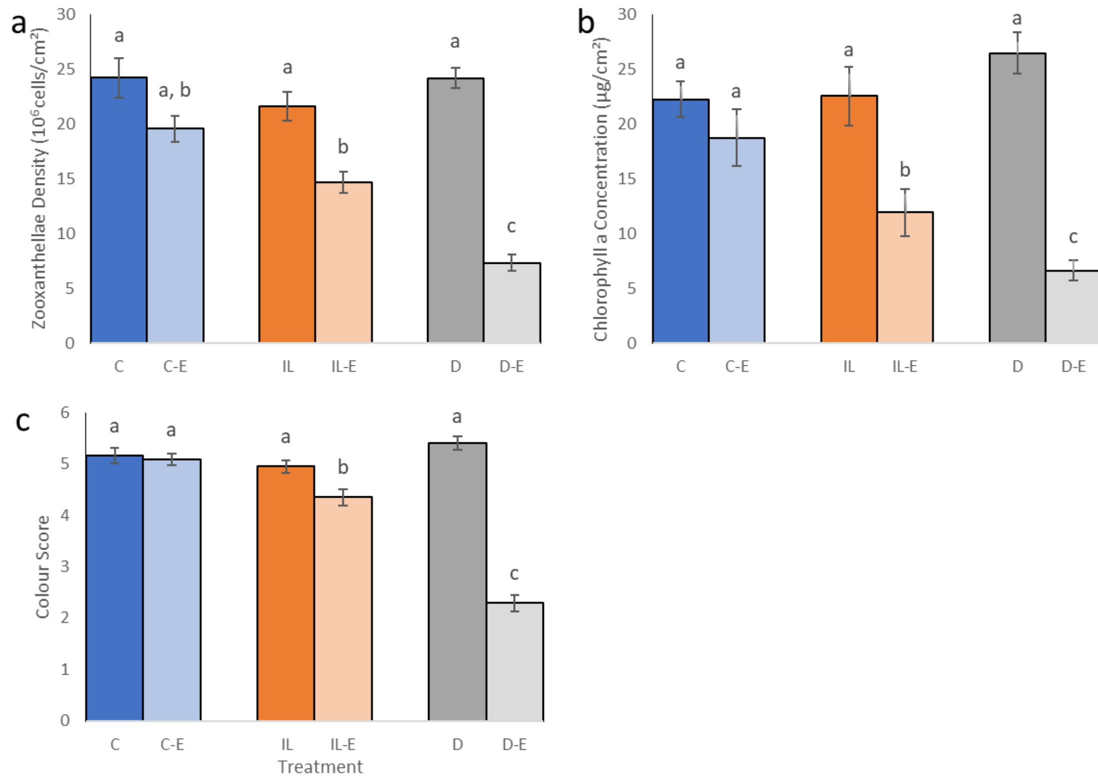


Fig. 4.1: Zooxanthellae Density (a), and Chlorophyll a Concentration (b), Colour Score (c), across Control (C), Intermittent Light (IL) and Dark (D) treatment at the beginning of the experiment compared to the end respectively (C-E, IL-E, D-E). Data are expressed as value ± SE. Significant differences between samples were evaluated trough one-way analysis of variance (ANOVA) followed by Tukey's pairwise test (b) or Kruskal-Wallis non-parametric statistical test (a, c). Same letters above the graph indicate no significant difference in mean value

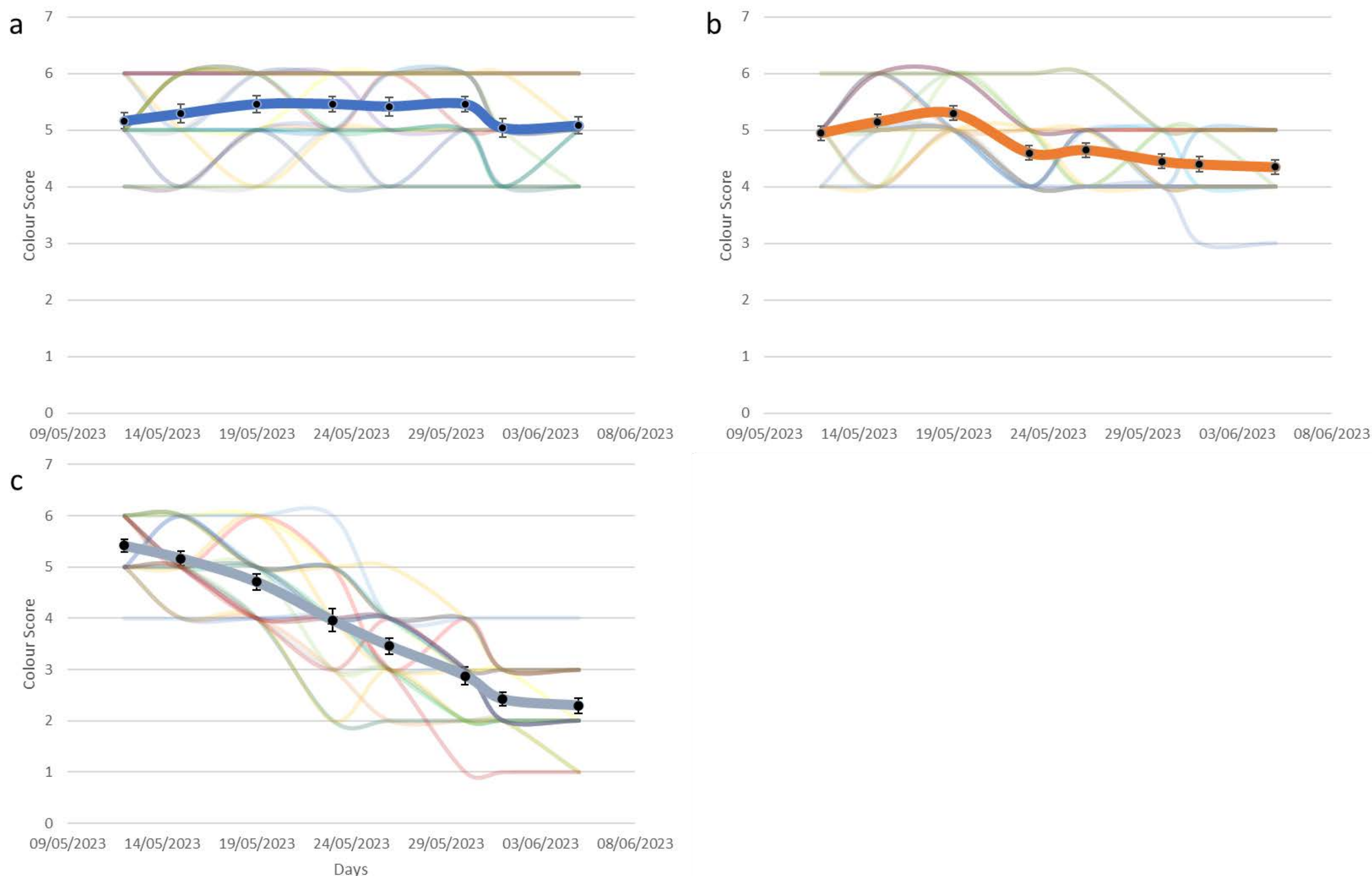


Fig. 4.2: Colour score of each fragment in Control (a), Intermittent Light (b), and Dark (c) Treatment recorded twice a week throughout the experiment. The bigger and brighter line represents the mean colour score of the entire treatment, the thinner and paler ones the colour score of every fragment in the treatment

4.2 Photo-Physiology Measurements

4.2.1 Maximum Quantum Yield (MQY)

Maximum quantum yield was measured at the beginning and at the end of the experiment for all 68 fragments of *P. speciosa*. Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 12.265$, $p < 0.05$, Fig. 4.3a).

For Control treatment no significant difference was observed between the start (C) and the end (C-E). Moreover, Intermittent Light treatment no significant difference was observed between the start (IL) and the end (IL-E). Finally, Dark treatment significant difference was observed between the start (D) and the end (D-E). There was a decrease of 17% ($P < 0.01$) decreasing from a mean value of 0.398 ± 0.018 to a mean value of 0.327 ± 0.020.

4.2.2 Effective Quantum Yield (EQY)

Effective quantum yield was measured at the beginning and at the end of the experiment for all 68 fragments of *P. speciosa*. Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 25.339$, $p < 0.01$, Fig. 4.3b).

For Control treatment no significant difference was observed between the start (C) and the end (C-E). Moreover, Intermittent Light treatment significant difference was observed between the start (IL) and the end (IL-E). There was an increase of 86% ($P < 0.01$) decreasing from a mean value of 0.220 ± 0.030 to a mean value of 0.408 ± 0.032. Finally, Dark treatment no significant difference was observed between the start (D) and the end (D-E).

4.2.3 Electron Transport Rate max (ETR_{max})

Electron Transport Rate max (ETR_{max}) was measured at the beginning and at the end of the experiment for all 68 fragments of *P. speciosa*. Significant differences were detected between the different treatments analysed (one-way ANOVA, $F(5, 127) = 26.841$, $P < 0.01$, Fig. 4.3c).

For Control treatment no significant difference was observed between the start (C) and the end (C-E). Moreover, Intermittent Light significant difference was observed between the start (IL) and the end (IL-E). There was a decrease of 50% ($P < 0.01$) decreasing from a mean value of 57.61 ± 2.61 to a mean value of 28.75 ± 3.13. Finally, Dark treatment significant difference was observed between the start (D) and the end (D-E). There was a decrease of 43% ($P < 0.01$) decreasing from a mean value of 59.64 ± 1.82 to a mean value of 33.97 ± 3.17.

4.2.4 Saturation Irradiance (E_k)

Minimum saturation irradiance (E_k) was measured at the beginning and at the end of the experiment for all 68 fragments of *P. speciosa*. Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 53.988$, $p < 0.01$, Fig. 4.3d).

For Control treatment significant difference was observed between the start (C) and the end (C-E). There was a decrease of 19% ($P < 0.01$) decreasing from a mean value of 224.77 ± 7.71 to a mean value of 182.00 ± 8.94. Moreover, Intermittent Light treatment significant difference was observed between the start (IL) and the end (IL-E). There was a decrease of 41% ($P < 0.01$) decreasing from a mean value of 223.01 ± 11.20 to a mean value of 131.80 ± 13.67. Finally, Dark treatment no significant difference was observed between the start (D) and the end (D-E).

4.2.5 Photochemical Efficiency (Alpha)

Photochemical efficiency (alpha) was measured at the beginning and at the end of the experiment for all 68 fragments of *P. speciosa*. Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 77.739$, $p < 0.01$, Fig. 4.3e).

For Control treatment significant difference was observed between the start (C) and the end (C-E). There was an increase of 15% ($P < 0.01$) increasing from a mean value of 0.25 ± 0.00 to a mean value of 0.28 ± 0.01. Moreover, Intermittent Light treatment significant difference was observed between the start (IL) and the end (IL-E). There was a decrease of 17% ($P < 0.01$) decreasing from a mean value of 0.26 ± 0.00 to a mean value of 0.22 ± 0.01. Finally, Dark treatment significant difference was observed between the start (D) and the end (D-E). There was a decrease of 53% ($P < 0.01$) decreasing from a mean value of 0.24 ± 0.01 to a mean value of 0.11 ± 0.01.

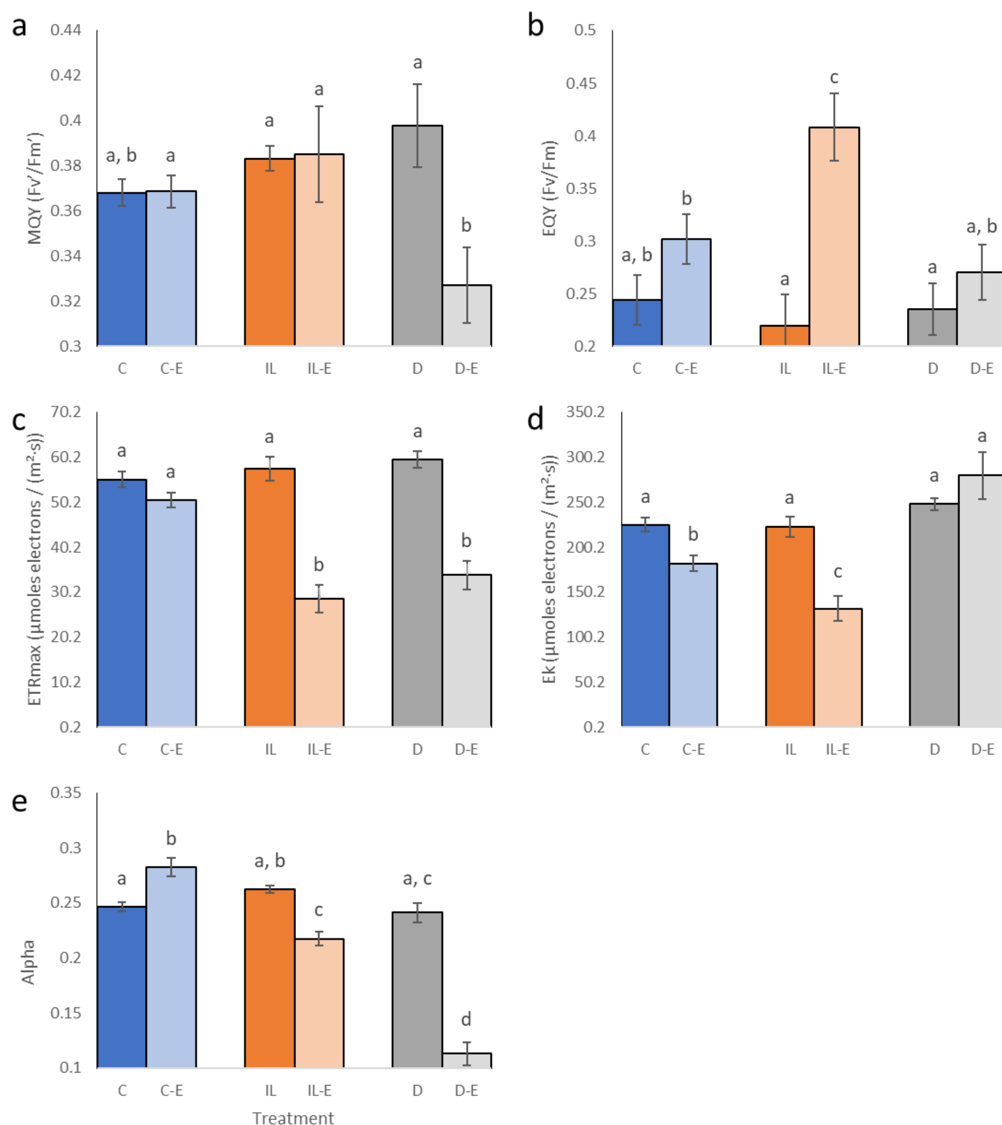


Fig. 4.3: Maximum Quantum Yield (MQY) (a), Effective Quantum Yield (EQY) (b), Electron Transport Rate max (ETR_{max}) (c), Light Saturation Parameter (E_k) (d), Photochemical Efficiency (alpha) (e), across Control

(C), Intermittent Light (IL) and Dark (D) treatment at the beginning of the experiment compared to the end respectively (C-E, IL-E, D-E). Data are expressed as value ± SE. Significant differences between samples were evaluated trough one-way analysis of variance (ANOVA) followed by Tukey's pairwise test (c) or Kruskal-Wallis non-parametric statistical test (a, b, d, e). Same letters above the graph indicate no significant difference in mean value

4.3 Ambient Measurements

4.3.1 Photosynthetic Active Radiation (PAR)

Photosynthetic active radiation (PAR) was recorded every day every 10 minutes in each tank for three weeks until the end of the experiment.

The light irradiation followed the normal circadian rhythms of 12 hours of light and 12 hours of shade (from 7:00 to 19:00). Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 123.283$, $p < 0.01$, Fig. 8.3). In particular, between Control and Intermittent Light ($P < 0.01$), between Intermittent Light and Dark ($P < 0.01$), and between Control and Dark ($P < 0.01$). The mean values recorded were calculated between 7:00 and 19:00, Control: 32.5 ± 1.2 µmol m^{-2} s^{-1}, Intermittent Light: 7.1 ± 0.6 µmol m^{-2} s^{-1}, and Dark: 1.7 ± 0.3 µmol m^{-2} s^{-1}.

4.3.2 Temperature

Temperature was recorded every day every 10 minutes in each tank for three weeks until the end of the experiment.

Significant differences were detected between the different treatments analysed (Kruskal-Wallis $\chi^2(2) = 30.158$, $p < 0.01$, Fig. 8.2). In particular, between Control and Intermittent Light ($P > 0.05$), between Intermittent Light and Dark ($P < 0.01$), and between Control and Dark ($P < 0.01$). in general, the temperature remained stable throughout the experiment in all the treatments. The mean values recorded were for Control: 30.90 ± 0.3 °C, for Intermittent Light: 30.89 ± 0.3 °C, and for Dark: 30.98 ± 0.2 °C. Significant differences in temperature were observed between the treatments.

4.4 RNA Extraction

A trial was conducted to see whether the protocol needed to be optimized before the real experiment sample were analysed. The goal was to produce high-quality RNA using *P. speciosa* fragments that had been kept in RNAlater at -80 °C, using agarose gel electrophoresis, the quality was evaluated. The first column's line is the marker, while the following lines are various *Pachyseris speciosa* samples. Well, distinct bands on the agarose gel indicate that high-integrity

RNA was extracted. The presence of these bands demonstrates that the RNA was successfully extracted from the samples. The ideal gel should also be devoid of any background DNA traces as proof of the high quality of the extracted material.

The first trial yielded degraded RNA that appeared as a long smear on the gel. No distinct bands could be seen. The smear in the high molecular weight region is also indicative of genomic DNA contamination (Fig. 4.4a). In the second trial a lower and consistent amount of starting material was used to start the protocol (200 mg). The extraction procedure was performed on ice as far as possible, avoiding RNA degradation. Fig. 4.4b demonstrates the improved quality of the RNA extraction, showing clear bands and a reduced DNA background noise.

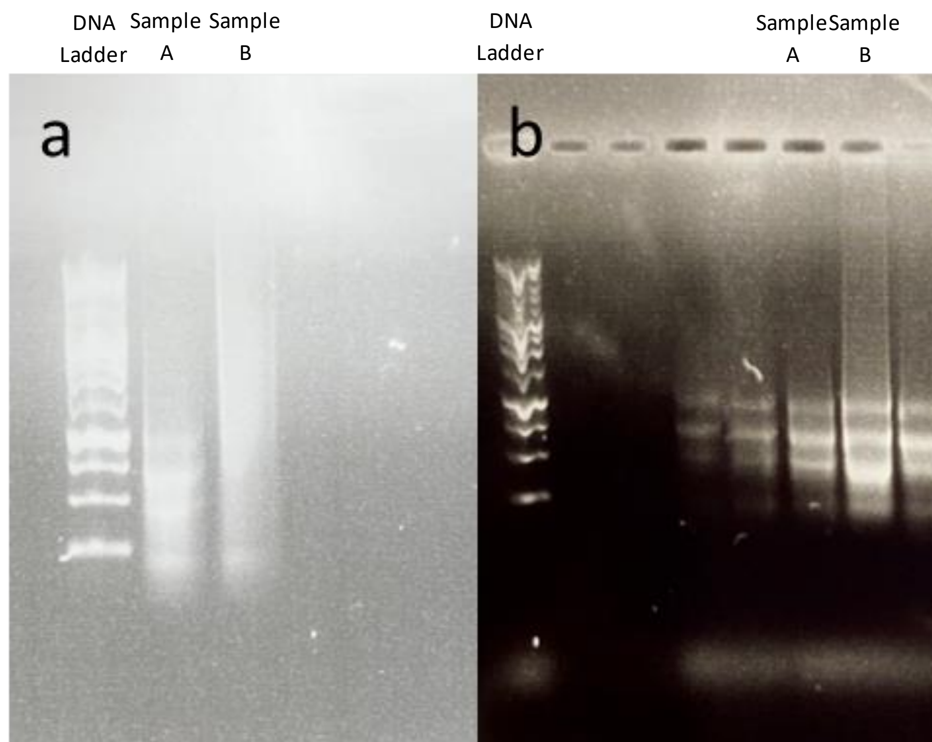


Fig. 4.4: Comparison between RNA extraction protocols (a) first trial (b) second trial

5. DISCUSSION

Corals thrive in a narrow range of salinity, light, and temperature values to survive. This sensitive organism becomes stressed by a small change in these parameters, and as a result, it expels its photosynthetic symbiont from its cells (Monroe et al., 2018) that can provide up to 90% of its required energy (Kaiser et al., 2005). Due to global changes and anthropogenic stresses, especially coming from increased sedimentation caused by sea-level rise and land reclamation, short- and long-term studies have been conducted to understand how corals are able to cope with these adverse conditions. It has been reported that ongoing land reclamation activities and the stress caused by it may have resulted in the extinction of some vulnerable species in Singapore's reefs (Poquita-Du et al., 2019). The P/R ratio is a dimensionless number which provides an estimate of the degree to which algal production of organic material exceeds plant plus animal consumption (Coles and Jokiel, 1977). High turbidity can reduce the P/R ratio by increasing respiration while decreasing photosynthesis, making corals more vulnerable to infections and bleaching (Anthony and Fabricius, 2000; Anthony and Connolly, 2004, Anthony and Connolly, 2007). Some corals can photoacclimate by increasing the concentrations of photosynthetic pigments and/or symbiont densities to deal with declines in light availability (Rogers, 1979; Dubinsky et al., 1984). This causes a measurable increase in the fluorescence yield and a decrease in the saturation irradiance (Anthony and Fabricius, 2000; Te, 2001).

In this experiment, Intermittent Light and Dark treatments presented statistically significant decreases in zooxanthellae density, chlorophyll a concentration, and colour score. Since in those treatments corals were exposed respectively to 7.1 ± 0.6 µmol m^{-2} s^{-1}, and 1.7 ± 0.3 µmol m^{-2} s^{-1} (PAR), this confirms that if low light conditions become too pressing, coral cells react by expelling symbiotic cells, leading to a decrease in symbiont density, loss of colouration, and chlorophyll concentration (Titlyanov et al., 2001; Browne et al., 2015, Oakley et al., 2018). Since for the Control treatment a non-significant decrease in zooxanthellae density, chlorophyll concentration and colour was observed, we can state that these two treatments were not influenced by the high water temperatures measured during the experiment. Additionally, interesting results were found for photo-physiological parameters. In fact, in Intermittent Light treatment there was a consistent and significant increase (+ 86%, $p < 0.01$) in EQY, and in parallel a statistically significant decrease in ETR_{max}, E_k, and alpha was also observed. This suggests that *Pachyseris speciosa* under this treatment was able to upregulate photosynthetic efficiency, coping with the new adverse conditions (also documented by Browne et al., 2014). Doing so, the coral was able to take the maximum advantage from the only two days of full light in a week. This is confirmed by the significant decrease in E_k, meaning that *P. speciosa* required much less light to saturate its

photosystems, resulting in an overall higher efficiency; this behaviour has also been observed by Anthony and Fabricius, 2000; and Te ,2001. In particular, in this first study *Goniastrea retiformis* and *Porites cylindrica* showed a decrease in E_k under shaded conditions, and the researchers concluded that this was a sign of photoacclimation. Moreover, Statton et al., 2018 observed a decrease in ETR_{max} under shading conditions, suggesting a potential acclimation of the coral. ETR_{max} and E_k are two parameters strictly connected to light availability; a decrease in ETR_{max} and E_k was also observed in corals with increasing depth (Hennige et al., 2008; Lesser et al., 2010), In parallel, MQY of *P. speciosa* nubbins remained unchanged, suggesting that the total photosynthetic capacity of the coral remained constant throughout the experiment. This confirms that this specific coral has a plastic photosynthetic mechanism that allows it to cope with acute low-light conditions. It is also interesting to note that EQY nearly reached MQY levels in Intermittent Light treatment, therefore, *P.* speciosa photosystems reached nearly their maximum photosynthetic rate even if not dark acclimated. Additionally, the significant correlation found between zooxanthellae density and colour suggests that the density of symbionts in the coral is closely coupled with the intensity of its colour (Fig. 8.5). Therefore, in our study, colour could be considered a good indicator of zooxanthellae density in *P. speciosa*. On the other hand, chlorophyll a concentration seems to be not as accurate as zooxanthellae density in estimating colour score, probably because some algal formation on the fragments could have influenced the spectrophotometric result, but it still remains a considerable indicator (Fig. 8.6). The same could be valid to correlate chlorophyll a concentration to zooxanthellae density (Fig. 8.4). Alongside this, colour seems also to be a good estimator of photochemical efficiency (Alpha) (Fig. 8.10) whereas ETR_{max} shows a moderate positive correlation with zooxanthellae density and colour score (Fig. 8.7; 8.8) and also alpha shows a moderate positive correlation with zooxanthellae density (Fig. 8.9). Previous experiments, show that algal or pigments density increase in corals under low light conditions, allowing photo-acclimation (Falkowski and Dubinsky, 1981; Titlyanov et al., 2001). Additionally, Dobson et al., (2021) highlighted that chlorophyll a concentration was lower in high-light conditions compared to lower ones; this has been considered as an acclimation process. However, in this thesis experiment, a decrease in chlorophyll a concentration and in zooxanthellae density was recorded in both Intermittent Light and Dark treatments. Alongside this, photo-physiology parameters showed acclimation responses. It is possible that as a compensation, *P. speciosa* increased heterotrophy, as documented for other corals (Anthony and Fabricius, 2000; Treignier et al., 2008) and that it could have regulated other photo-pigments not measured in our experiment, allowing the increased photosynthetic efficiency. On the other hand, photochemical capacity decreased significantly, by contrast in Ziegler et al., 2014 article significantly increased with depth. It is feasible that it decreased because photosynthesis energy apport was partially replaced by heterotrophy

and this last one was able not only to sustain all the biological functions of the coral, but also to allow its acclimation.

The Dark treatment presented a remarkable decline in all bleaching parameters as mentioned before (zooxanthellae density, chlorophyll a concentration, and colour score). Additionally, the significant decrease observed in MQY, ETR_{max}, and Alpha, clearly states that corals were experiencing stress. In fact, MQY decrease indicates that fragments under this treatment were depleted in photosynthetic capacity. It is possible that similarly to Intermittent Light treatment corals were able to shift their metabolic apport toward heterotrophy. However, in this case, it was not enough to let the coral acclimate. This is also confirmed by the stability of EQY. In fact, unlike in Intermittent Light treatment, fragments were not able to adapt their photosynthetic efficiency to the new adverse environment, leaving them without enough energy to recover. Moreover, the decrease in ETR_{max}, so the ability to convert light energy into chemical energy, was not followed by a decrease in E_k. This suggests that these fragments required the same amount of light to saturate their photosystems as at the beginning of the experiment, when they were completely healthy. Nonetheless, all *P. speciosa* fragments were able to survive for four weeks even in complete darkness, suggesting that this species is highly adaptable to new low-light conditions even for a considerable amount of time.

Interestingly, we observed the steeper change in colour score after 8 days from the beginning of the experiment, in particular in the Intermittent Light treatment. Since this rapid decline in colour was followed by a gentler decline in it, it is feasible that this is a dynamic of acclimation of fragments, as seen in other experiments (Titlyanov et al., 2001). We can presume that, chlorophyll a concentration and zooxanthellae density decreased simultaneously and that *P. speciosa* may be able to adapt at a photo-physiological level in response to temporary changes in light conditions in the habitat, such as increased water turbidity or increased run-off, in a relatively short time.

Finally, the Control treatment did not show a statistically significant increase or decrease in parameters between the beginning and the end, except for E_k and Alpha. Specifically, the decrease in zooxanthellae density, chlorophyll a concentration, colour score, and ETR_{max}, even if not statistically significant, could be due to the abnormally high water temperature recorded during the month of May around St. John's Island (30.8 ± 0.0 °C (https://ombak.mesn.sg/)) reaching a peak of 31.6 °C on the 31st May 2023 (toward the end of the experiment) (Fig 8.2a; 8.2b). Moreover, the increase in EQY could be an indicator of acclimation to a new slightly different light environment, since before corals were kept in an indoor aquarium with artificial illumination, even if mimicking the actual PAR and day-night cycle of Singapore's reefs.

No fragments mortality was observed at the end of the experiment, therefore, *P. speciosa* was able to survive for four weeks even in complete darkness. Since light availability in Singapore's

reefs strongly declines with depth and it is heavily impacted by land reclamation and dissolved organic matter in the water column (Champ et al., 1977; Pickering et al., 2015), the fact that all of the fragments were able to survive is another indicator that *P. speciosa* may develop adaptations which enable it to persist in Singapore's reef challenging conditions (Chou, 1988b; Dikou and van Woesik, 2006; Guest et al., 2016), even during extreme events. In addition, to survive in turbid environments it is already known that *P. speciosa* can produce sediment-trapping mucous sheets effective against high sediment in the water (Bessell-Browne et al., 2017). Moreover, its laminar growth strategy, allows it to win the competition with other corals in less illuminated reefs (Chow et al., 2019); this present study brings evidence to low light adaptation in *P. speciosa* at photo-physiological level.

PAM fluorescence measurements are widely used in coral studies. However, this technique has some limitations that should be considered when planning studies or analysing data. For instance, sedentary animals are constantly exposed to spectrum shifts, even in the short term, irradiance levels influence the efficiency of photosynthetic processes and, subsequently, the results of these measurements. In this experiment, this limitation was addressed by maintaining the same light conditions for all the fragments while measuring photo-physiological data. The measurement may also be influenced by the organism's morphology. Therefore, studies of chlorophyll fluorescence must be combined with traditional bleaching assessment techniques for a more accurate assessment. Moreover, a more frequent data collection would have given a more precise data set and could have further helped in the interpretation of the results, understanding the turning points in coral photo-physiology status. Additionally, the colour score is a visual assessment. The usage of a computer program would have helped in obtaining more accurate results.

In addition to the photo-physiological analyses, transcriptomics analyses were also performed to complement the data. However, being a time demanding process, is still ongoing, and the results could not be inserted into this dissertation. Transcriptomic data could have helped in better understanding acclimation processes in *P. speciosa* and in assessing their stress level.

Finally, the study was performed only on the species *Pachyseris speciosa*, therefore, further studies need to be addressed considering different coral species particularly present in Singapore's reefs such as *Goniastrea pectinata* or *Porites lutea*. Future studies could also extend the experimental period beyond four weeks to determine the survival threshold in complete darkness for *Pachyseris speciosa* and/or monitor the corals following the removal of the extreme low-light conditions to determine recovery processes following such stress. Furthermore, transcriptomic responses could be considered in addition to photo-physiology. Additionally, analysis of antioxidant enzymes and of P/R ratio could be considered in further studies to better understand adaptation mechanisms and heterotrophy.

6. CONCLUSION

This study demonstrated that the coral *Pachyseris speciosa* is able to acclimate and survive for up to four weeks being subjected to extreme low light conditions. In particular, the intermediate treatment, Intermittent Light, in which the coral fragments were subjected to two days of light and five days of complete darkness, showed acclimation signals through the following parameters: increased EQY, and decreased ETR_{max}, E_k, and alpha. Although all coral fragments in Dark treatments survived till the end of the four-week experiment, the significantly reduced zooxanthellae density and chlorophyll a concentration as well as colour clearly indicated stress and bleaching. Nevertheless, this study has demonstrated that *P. speciosa* has the ability to cope with low-light conditions at a photo-physiological level, becoming more efficient by increasing photosynthetic efficiency, as seen in particular in Intermittent Light treatment. These findings suggest that this coral is able to quickly adapt to abnormal low-light conditions at a photo-physiological level.

This thesis provides some scientific basis to fill knowledge gaps on *Pachyseris speciosa* adaptation and photo-physiological responses to extreme low-light conditions or even complete darkness. Specifically, it is fundamental to understand that this species can resist to increasing low-light penetration in the water column due to increased sedimentation and that *P. speciosa* can be relied on to be transplanted in extreme low-light conditions (under 6 m around Singapore) helping in the conservation of Singapore's reefs ecosystem even considering the possible worsening of water conditions due to anthropogenic stress or climate change.

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8. SUPPLEMENTARY

21444328	y = 0.8051x + 11.49	T1
21444331	y = 0.8978x + 16.158	T3
21444322	y = 0.8334x + 15.189	T5
21444325	y = 0.7761x + 9.0117	T8
21444327	y = 0.8641x + 15.365	T10
21444330	y = 0.9632x + 6.7056	T12

Fig. 8.1: Identification numbers, and calibration factors of the HOBO loggers with respective tank number

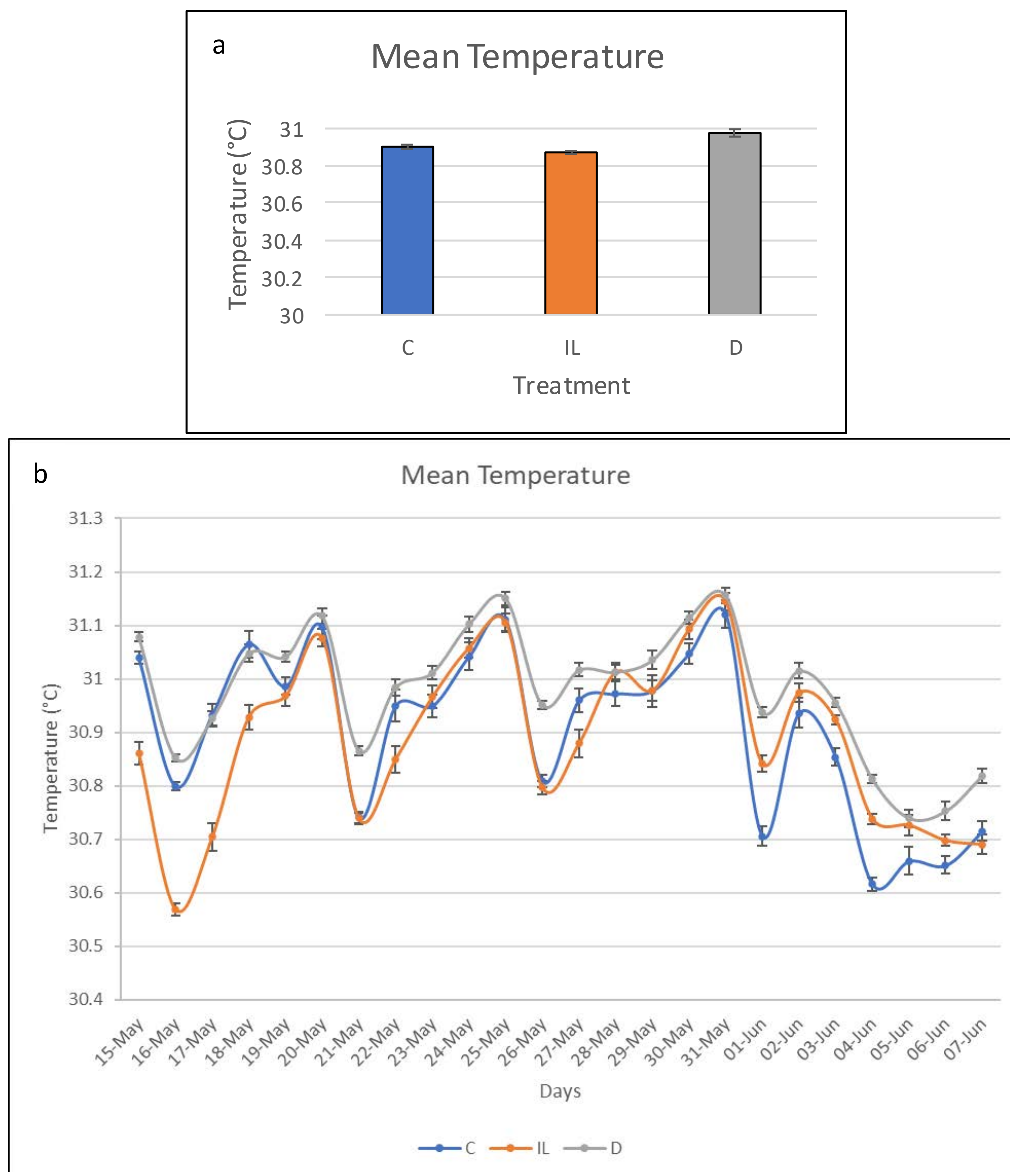
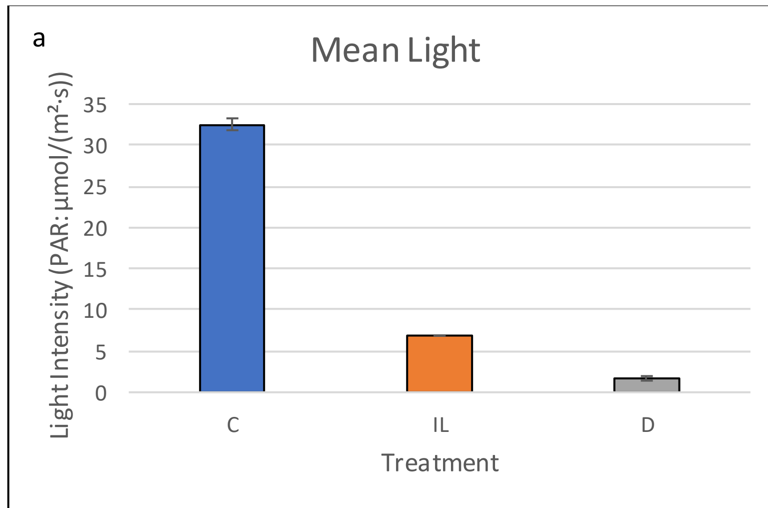


Fig. 8.2: Mean water temperature divided by treatment. a) Overall mean water temperature, b) Daily mean surface temperature



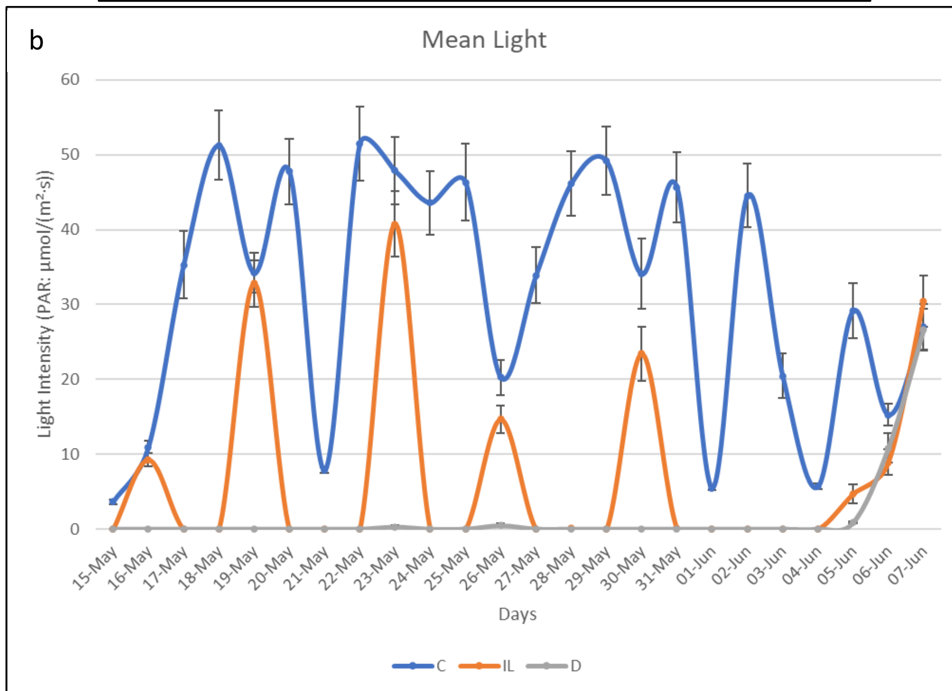


Fig. 8.3: Mean light intensity divided by treatment. a) Overall mean light intensity, b) Daily mean light intensity

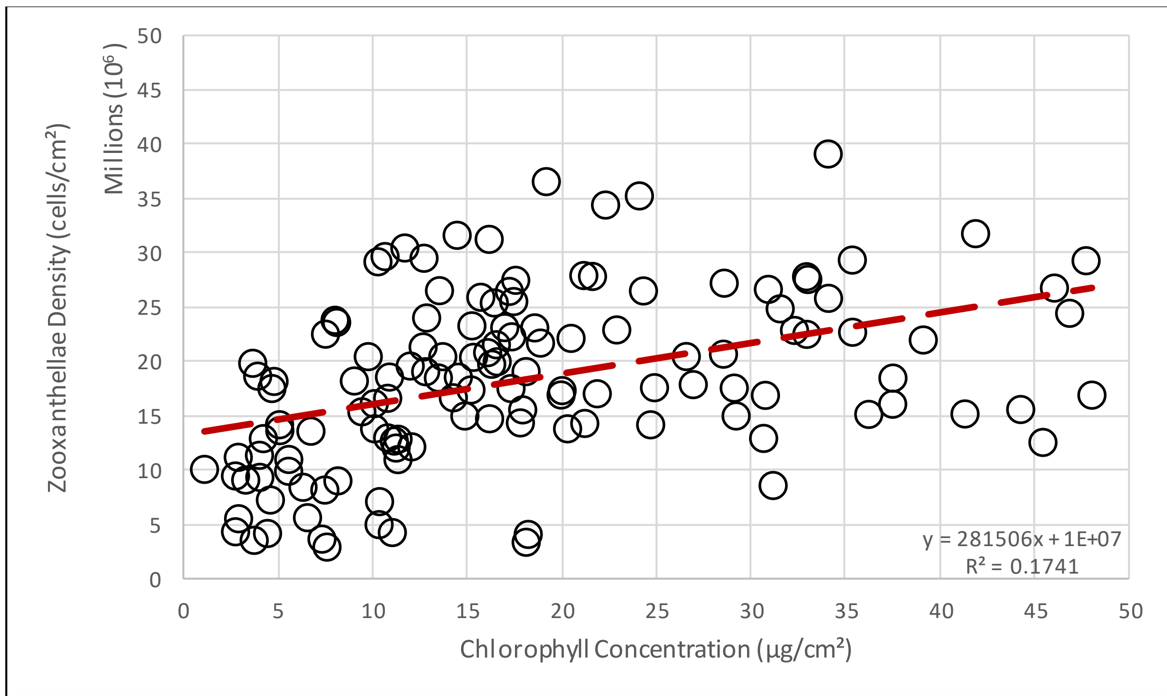


Fig. 8.4: Pearson product-moment correlation between chlorophyll a concentration and zooxanthellae density. Moderate, positive correlation ($r = .417$, $n = 131$, $p < 0.01$)

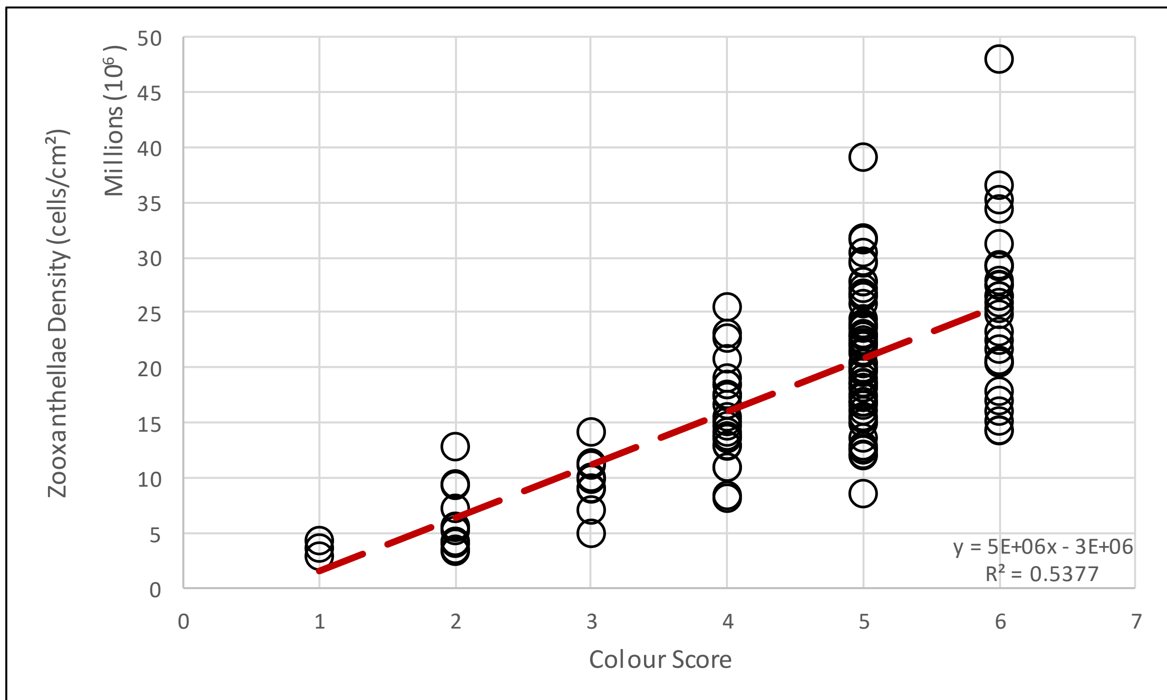


Fig. 8.5: Pearson product-moment correlation between zooxanthellae density and colour. Strong, positive correlation ($r = .733$, $n = 135$, $p < 0.01$)

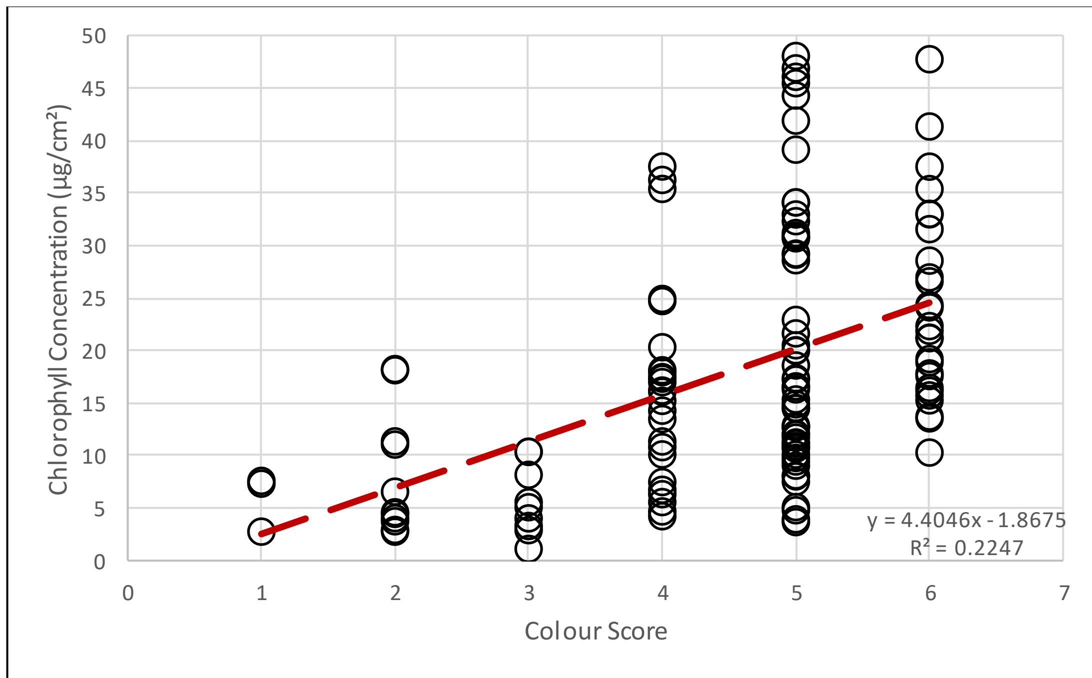


Fig. 8.6: Pearson product-moment correlation between chlorophyll a concentration and colour. Moderate, positive correlation ($r = .474$, $n = 132$, $p < 0.01$)

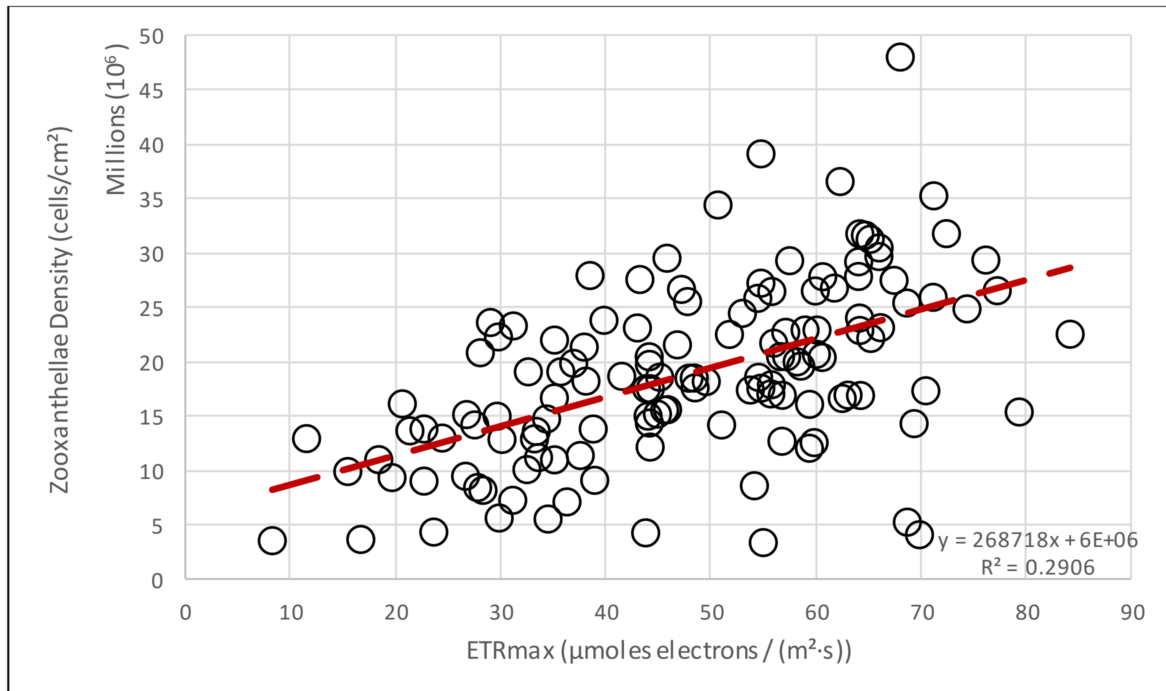


Fig. 8.7: Pearson product-moment correlation between ETR_{max} and zooxanthellae density. Moderate, positive correlation ($r = .539$, $n = 132$, $p < 0.01$)

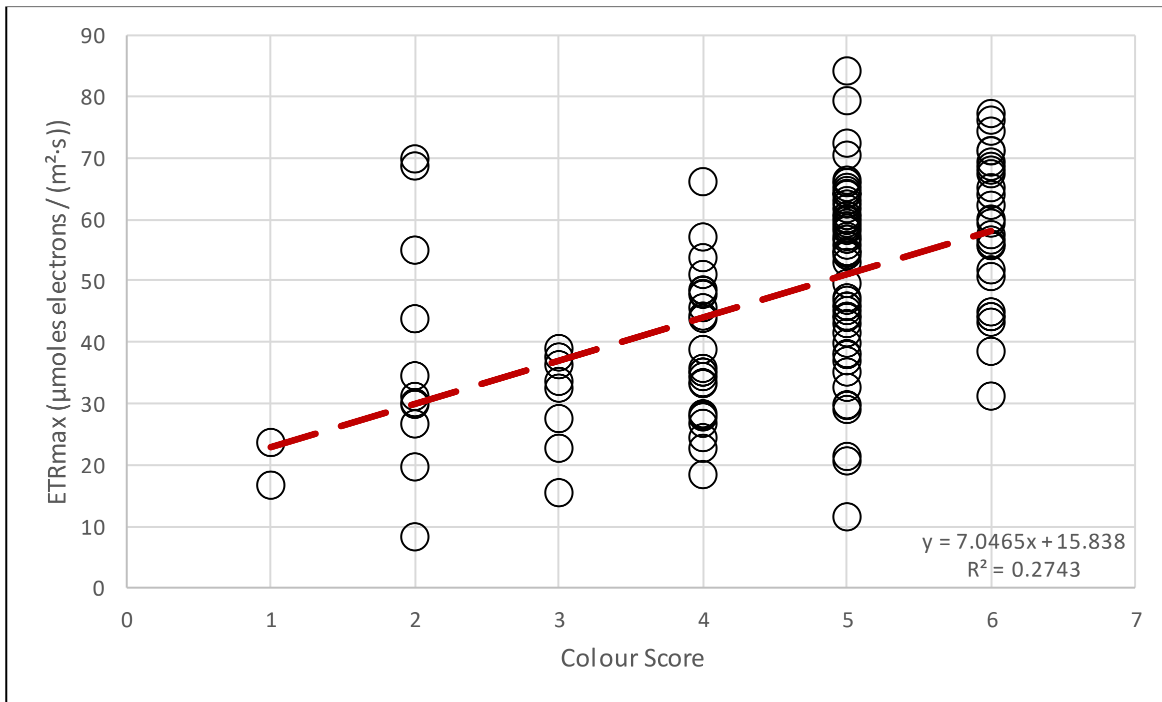


Fig. 8.8: Pearson product-moment correlation between colour and ETR_{max}. Moderate, positive correlation ($r = .524$, $n = 133$, $p < 0.01$)

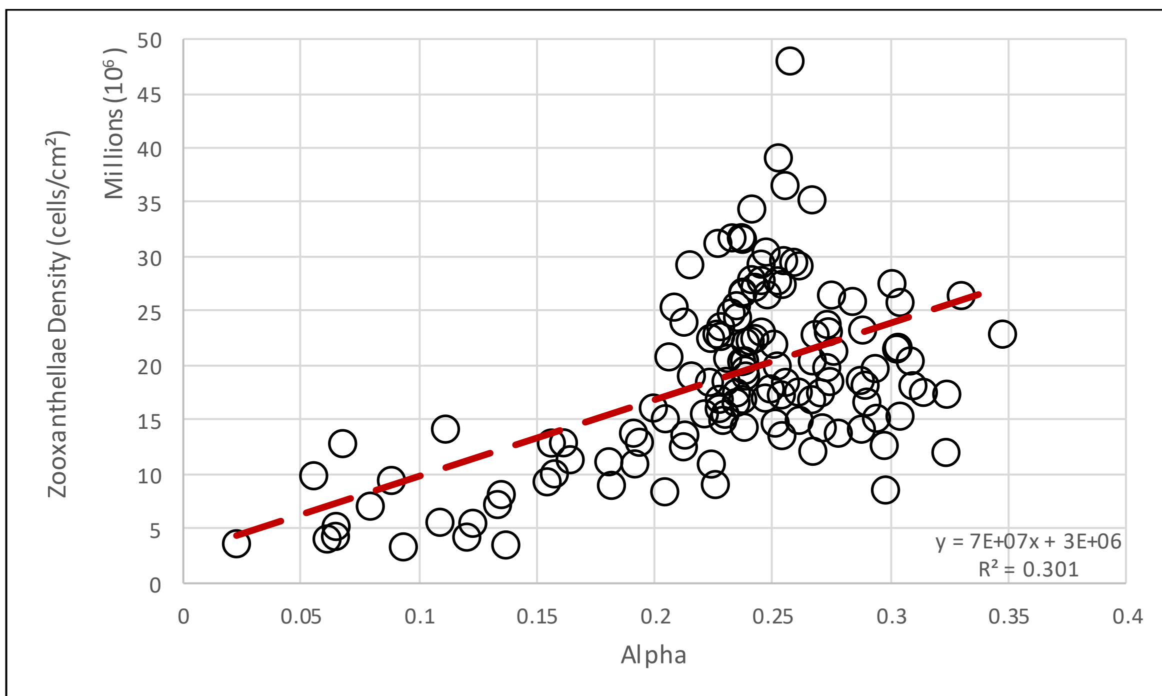


Fig. 8.9: Pearson product-moment correlation between alpha and zooxanthellae density. Moderate, positive correlation ($r = .549$, $n = 132$, $p < 0.01$)

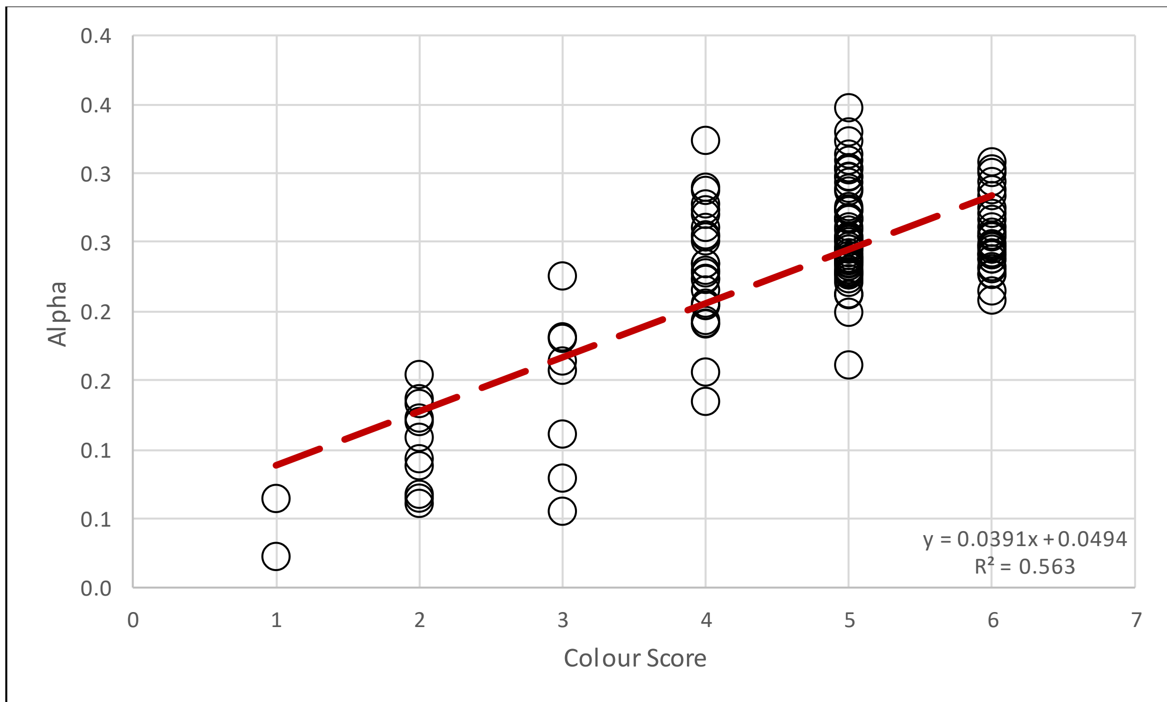


Fig. 8.10: Pearson product-moment correlation between colour and alpha. Strong, positive correlation (r = .750, n = 133, p < 0.01)